

FORM PTO-1390 (Modified)
(REV 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES
DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. 371

20-02

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR

not assigned 10/069799

INTERNATIONAL APPLICATION NO.
PCT/AU00/01048

INTERNATIONAL FILING DATE
31 August 2000

PRIORITY DATE CLAIMED
31 August 1999

TITLE OF INVENTION

Vaccine Antigens of Moraxella

APPLICANT(S) FOR DO/EO/US

FARN, Jacinta, STRUGNELL, Richard and TENNENT, Jan

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
 - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
 - b. ☒ has been communicated by the International Bureau.
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
 - a. ☐ is attached hereto.
 - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
 - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
 - b. ☐ have been communicated by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☐ Other items or information:

Page 2 of 2

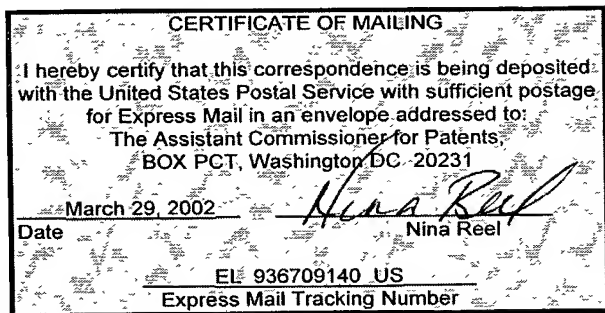
100672747 ... 100672747 #4/B

JCO2 Rec'd PCT/PTO 29 MAR 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: :
Farn et al. :
Serial No.: 10/069,799 :
Filed: February 28, 2002 :
For: VACCINE ANTIGENS OF MORAXELLA

Group Art Unit: not assigned
Examiner: not Assigned



SECOND PRELIMINARY AMENDMENT

Asst. Commissioner for Patents
Box PCT
Washington, D.C. 20231

Sir:

Please amend the above-referenced application as follows:

In the specification:

Please insert the following as the first paragraph of the specification:

--CROSS REFERENCE TO RELATED APPLICATIONS

This application is a National Stage Application of PCT International Application No. PCT/AU00/01048, filed on August 31, 2000 which claims priority to Australian Patent Application No. PQ2571, filed on August 31, 1999, both of which are incorporated herein, by reference, in their entirety.--

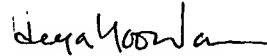
REMARKS

The application has been amended to claim priority to related patents under 37 C.F.R. 1.78.

CONCLUSION

It is believed that no fee is due with the submission of this Second Preliminary Amendment. However, if this is incorrect, please charge the required fee to Deposit Account No. 07-1969.

Respectfully submitted,



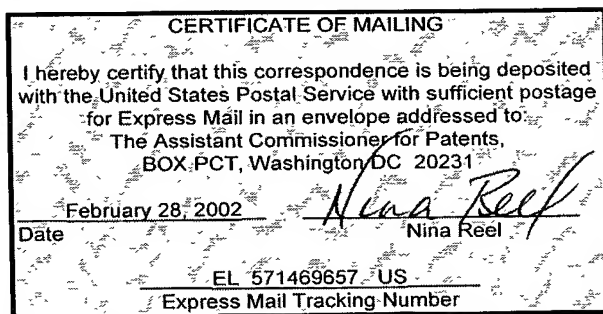
Heeja Yoo-Warren, Ph.D.
Reg. No. 45,495

GREENLEE, WINNER AND SULLIVAN, P.C.
5370 Manhattan Circle, Suite 201
Boulder, CO 80303
Telephone: (303) 499-8080
Facsimile: (303) 499-8089
E-mail: winner@greenwin.com

Attorney docket No. 20-02
nnr: March 29, 2002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: :
Farn et al. : Group Art Unit: not assigned
Serial No.: not assigned : Examiner: not Assigned
Filed: February 27, 2002 :
For: VACCINE ANTIGENS OF MORAXELLA



PRELIMINARY AMENDMENT

Asst. Commissioner for Patents
Box PCT
Washington, D.C. 20231

Sir:

This amendment accompanies a filing of a U.S. National Stage application under 35 U.S.C. 371. Filing fees for the National Stage application have been calculated based on this amendment.

Please amend the application as follows:

In the Claims:

Please cancel claims 1-38 and enter the following new claims 39-84.

- 39. A polypeptide having an amino acid sequence as set out in SEQ ID NO:1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.
40. The polypeptide, as claimed in claim 39, having a sequence of at least 70% identity with the sequence shown in SEQ ID NO:1 from amino acid 37 to 1114.

41. The polypeptide, as claimed in claim 39, having a sequence of at least 80% identity with the sequence shown in SEQ ID NO:1 from amino acid 37 to 1114.
42. The polypeptide, as claimed in claim 39, having a sequence of at least 90% identity with the sequence shown in SEQ ID NO:1 from amino acid 37 to 1114.
43. A polypeptide, as claimed in claim 39, having protease activity.
44. A nucleic acid molecule comprising a sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.
45. A nucleic acid molecule comprising a sequence as set out in SEQ ID NO:2 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.
46. The nucleic acid molecule, as claimed in claim 45, comprising a sequence having at least 70% identity with the sequence shown in SEQ ID NO:2.
47. The nucleic acid molecule, as claimed in claim 45, comprising a sequence having at least 80% identity with the sequence shown in SEQ ID NO:2
48. The nucleic acid molecule, as claimed in claim 45, comprising a sequence having at least 90% identity with the sequence shown in SEQ ID NO:2.
49. A composition for use in raising an immune response in an animal comprising a polypeptide having an amino acid sequence as set out in SEQ ID NO:1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.
50. A composition for use in raising an immune response in an animal, comprising a nucleic acid sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:1 from amino acid 37 to 1114, or a sequence having at least

50% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.

51. A polypeptide having an amino acid sequence as set out in SEQ ID NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.
52. The polypeptide, as claimed in claim 51, having a sequence of at least 70% identity with the sequence shown in SEQ ID NO:3 from amino acid 26 to 616.
53. The polypeptide, as claimed in claim 51, having a sequence of at least 80% identity with the sequence shown in SEQ ID NO:3 from amino acid 26 to 616.
54. The polypeptide, as claimed in claim 51, having a sequence of at least 90% identity with the sequence shown in SEQ ID NO:3 from amino acid 26 to 616.
55. The polypeptide, as claimed in claim 51, having lipase activity.
56. A nucleic acid molecule comprising a sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.
57. A nucleic acid molecule comprising a sequence as set out in SEQ ID NO:4 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.
58. The nucleic acid molecule, as claimed in claim 57, comprising a sequence having at least 70% identity with the sequence shown in SEQ ID NO:4.
59. The nucleic acid molecule, as claimed in claim 57, comprising a sequence having at least 80% identity with the sequence shown in SEQ ID NO:4.
60. The nucleic acid molecule, as claimed in claim 57, comprising a sequence having at least 90% identity with the sequence shown in SEQ ID NO:4.

61. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide having an amino acid sequence as set out in SEQ ID NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.
62. A composition for use in raising an immune response in an animal, the composition comprising a nucleic acid sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof and optionally a carrier and/or adjuvant.
63. A polypeptide having an amino acid sequence as set out in SEQ ID NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.
64. The polypeptide, as claimed in claim 63, having a sequence of at least 70% identity with the sequence shown in SEQ ID NO:5.
65. The polypeptide, as claimed in claim 63, having a sequence of at least 90% identity with the sequence shown in SEQ ID NO:5.
66. The polypeptide, as claimed in claim 63, having haemolysin activity.
67. A nucleic acid molecule comprising a sequence encoding a polypeptide having an amino acid sequence as set out in SEQ ID NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.
68. A nucleic acid molecule comprising a sequence as set out in SEQ ID NO:6 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.
69. The nucleic acid molecule, as claimed in claim 68, comprising a sequence having at least 70% identity with the sequence shown in SEQ ID NO:6.

77. The composition, as claimed in claim 74, wherein the *Moraxella* is *M. bovis* or *M. catarrhalis*.
78. The composition, as claimed in claim 75, wherein the *Moraxella* is *M. bovis* or *M. catarrhalis*.
79. The composition, as claimed in claim 76, wherein the *Moraxella* is *M. bovis* or *M. catarrhalis*.
80. The composition, as claimed in claim 74, wherein the *Moraxella* is *M. bovis*.
81. The composition, as claimed in claim 75, wherein the *Moraxella* is *M. bovis*.
82. The composition, as claimed in claim 76, wherein the *Moraxella* is *M. bovis*.
83. An antibody raised against a polypeptide selected from the group consisting of a polypeptide as given in SEQ ID NO:1 from amino acid 37 to 1114 or a functional fragment thereof, SEQ ID NO:3 from amino acid 26 to 616 or a functional fragment thereof, and SEQ ID NO:5 or a functional fragment thereof.
84. The antibody of claim 83 wherein said polypeptide has at least 50% identity to the polypeptide as given in SEQ ID NO:1 from amino acid 37 to 1114 or a functional fragment thereof, SEQ ID NO:3 from amino acid 26 to 616 or a functional fragment thereof, and SEQ ID NO:5 or a functional fragment thereof.--

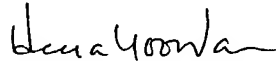
REMARKS

The claims of the international application have been replaced to correct formalities.
This amendment does not add new matter to the specification.

CONCLUSION

It is believed that no fee is due with the submission of this Preliminary Amendment.
However, if this is incorrect, please charge the required fee to Deposit Account No.
07-1969.

Respectfully submitted,



Heeja Yoo-Warren, Ph.D.
Reg. No. 45,495

GREENLEE, WINNER AND SULLIVAN, P.C.
5370 Manhattan Circle, Suite 201
Boulder, CO 80303
Telephone: (303) 499-8080
Facsimile: (303) 499-8089
E-mail: winner@greenwin.com

Attorney docket No. 20-02
nnr: February 28, 2002

Vaccine antigens of Moraxella

FIELD OF THE INVENTION

The present invention relates to antigens of *Moraxella*, in particular, *Moraxella bovis*, nucleic acid sequences encoding these antigens and
5 formulations for use in raising an immune response against *Moraxella*.

BACKGROUND OF THE INVENTION

Infectious bovine keratoconjunctivitis (IBK) is an economically important disease of cattle caused by the Gram-negative coccobacillus
10 *Moraxella bovis*. More commonly known as pinkeye, IBK is a highly contagious ocular infection which may range from mild conjunctivitis to severe ulceration, corneal perforation and blindness. Therapeutic and preventative measures have limited success in controlling IBK and a vaccine which will prevent the disease is required. A number of factors contribute to
15 the virulence of the organism, the two most important attributes so far identified are the expression of pili, and the ability to produce haemolysin. Seven different serogroups of *M. bovis* strains isolated in Australia, Great Britain and the USA have been characterised, based on pilus types (1). An efficacious pilus-based vaccine must contain a sufficient quantity of pili from
20 all seven serotypes to be fully protective, because of a lack of cross protection between serotypes (2, 3). Expression of all seven pilus serotypes at levels high enough to be useful for commercial vaccine preparation has not been achieved.

The ideal vaccine candidate to stimulate protection against *M. bovis*
25 would be a molecule that is highly-conserved among all strains of this species. Possible candidates are haemolysin, protease, lipase and/or phospholipase (4) enzymes produced by *M. bovis*. For example, a partially purified cell-free supernatant from one haemolytic strain of *M. bovis* has been shown to confer significant protection against heterologous, wild-type
30 challenge (5). The possibility that a haemolysin could be conserved across all seven serotypes of *M. bovis* makes it a potential vaccine candidate against IBK. However, researchers have so far been unable to either clone the gene encoding the haemolysin or purify the protein to homogeneity. Nevertheless, any or all of these molecules, alone or in combination, could prove useful for
35 the generation of an effective vaccine against IBK.

SUMMARY OF THE INVENTION

In a first aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 1 from
5 amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 1.

10 In a further preferred embodiment of the first aspect of the present invention the polypeptide has protease activity.

In a second aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the first aspect of the present invention.

15 In a third aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 2 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

In a preferred embodiment the nucleic acid molecule has a sequence of
20 at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 2.

In a fourth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising the polypeptide of the first aspect of the present invention or the nucleic acid
25 sequence of the second aspect of the present invention and optionally a carrier and/or adjuvant.

In a fifth aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 3 from
30 amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.

In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 3 from amino acid 26 to 616.

In a further preferred embodiment of the fifth aspect the polypeptide
35 has lipase activity.

In a sixth aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the fifth aspect of the present invention.

5 In a seventh aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 4 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

10 In a preferred embodiment the nucleic acid molecule has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 4.

15 In an eighth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising the polypeptide of the fifth aspect of the present invention or the nucleic acid sequence of the sixth aspect of the present invention and optionally a carrier and/or adjuvant.

In a ninth aspect the present invention consists in a polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.

20 In a preferred embodiment the polypeptide has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 5.

In a further preferred embodiment of the ninth aspect the polypeptide has haemolysin activity.

25 In a tenth aspect the present invention consists in a nucleic acid molecule, the nucleic acid molecule encoding the polypeptide of the ninth aspect of the present invention.

30 In an eleventh aspect the present invention consists in a nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO. 6 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

In a preferred embodiment the nucleic acid molecule has a sequence of at least 70%, more preferably at least 80% and most preferably at least 90% identity with the sequence shown in SEQ. ID. NO. 6.

35 In a twelfth aspect the present invention consists in a composition for use in raising an immune response in an animal, the composition comprising

the polypeptide of the ninth aspect of the present invention or the nucleic acid sequence of the tenth aspect of the present invention and optionally a carrier and/or adjuvant.

5 The term "functional fragment" as used herein is intended to cover fragments of the polypeptide which retain at least 10% of the biological activity of the complete polypeptide. In particular this term is used to encompass fragments which show immunological cross-reactivity with the entire polypeptide, eg ligands which react with the fragment also react with the complete polypeptide.

10 In a thirteenth aspect the present invention consists in a composition for use in raising an immune response in an animal directed against *Moraxella*, the composition comprising at least one polypeptide selected from the group consisting of the polypeptides of the first, fifth and ninth aspects of the present invention and optionally including an adjuvant or carrier.

15 In a preferred embodiment the composition includes the polypeptide of the ninth aspect of the present invention and either one of, or preferably both, the polypeptides of the first and fifth aspects of the present invention.

In a preferred embodiment the *Moraxella* is *M. bovis* or *M. catarrhalis*, most preferably *M. bovis*.

20 In a fourteenth aspect the present invention consists in an antibody raised against a polypeptide selected from the group consisting of the polypeptides of the first, fifth and ninth aspects.

25 As will be readily appreciated by the person skilled in this field the polypeptides and antibodies of the present invention and probes derived from the nucleotide sequences can be used as diagnostic reagents in determining *Moraxella*, in particular, *M. bovis* infection. For example, the polypeptides and antibodies can be used in ELISA based assays whilst the probes can be used in PCR based assays. The probes will be of a length to provide the required level of specificity and will typically be at least 18
30 nucleotides in length.

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements,
35 integers or steps.

BRIEF DESCRIPTION OF THE FIGURES

- Figure 1: Nucleotide and amino acid sequence of a protease from *M. bovis* Dalton 2d. A putative promoter sequence is singly underlined. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Putative transcription terminator sequences are indicated by inverted arrows. Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.
- Figure 2: Nucleotide and amino acid sequence of a lipase from *M. bovis* Dalton 2d. A putative promoter sequence is singly underlined. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Putative transcription terminator sequences are indicated by inverted arrows. Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.
- Figure 3: Heat stability of the lipase from *M.bovis* when expressed in its recombinant form (pMB1/MC1061). (Heating carried out at 90°C).
- Figure 4: Comparison of growth rate and expression levels of the lipase of *M.bovis* when in its (i) native form and (ii) recombinant form. The growth rate is shown as solid bars and the lipase expression levels as open diamonds.
- Figure 5: Nucleotide and amino acid sequence of the A subunit of the RTX toxin from *M. bovis* Dalton 2d. A putative ribosome binding site is shown in bold and underlined. A putative start codon is shown in bold. Upstream of the A subunit open reading frame is a portion of the coding region for the C subunit (nucleotide 1 to 195) (corresponding amino acid sequence shown in SEQ ID NO:8) and downstream of the A subunit is a small portion of the B subunit coding region (nucleotide 3080 to 3250) (corresponding amino acid sequence shown in SEQ ID NO:9) . Numbering for both the nucleotide and amino acid sequences are shown on the left hand side.

DETAILED DESCRIPTION OF THE INVENTION

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following non-limiting Examples.

General Molecular Biology

Unless otherwise indicated, the recombinant DNA techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present) and are incorporated herein by reference.

Protein Variants

Amino acid sequence variants can be prepared by introducing appropriate nucleotide changes into DNA, or by *in vitro* synthesis of the desired polypeptide. Such variants include, for example, deletions, insertions or substitutions of residues within the amino acid sequence. A combination of deletion, insertion and substitution can be made to arrive at the final construct, provided that the final protein product possesses the desired characteristics. The amino acid changes also may alter post-translational processes such as altering the membrane anchoring characteristics, altering the intra-cellular location by inserting, deleting or otherwise affecting the transmembrane sequences of the native protein, or modifying its susceptibility to proteolytic cleavage.

In designing amino acid sequence variants, the location of the mutation site and the nature of the mutation will depend on characteristic(s) to be modified. The sites for mutation can be modified individually or in

series, e.g., by (1) substituting first with conservative amino acid choices and then with more radical selections depending upon the results achieved, (2) deleting the target residue, or (3) inserting residues of other ligands adjacent to the located site.

5 A useful method for identification of residues or regions for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (*Science* (1989) **244**: 1081-1085). Here, a residue or group of target residues are identified (e.g., charged residues such as Arg. Asp, His, Lys, and Glu) and replaced by a neutral or negatively charged
10 amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the surrounding aqueous environment in or outside the cell. Those domains demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants. Thus, while the site for introducing an amino acid sequence variation is
15 predetermined, the nature of the mutation *per se* need not be predetermined. For example, to optimise the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at the target codon or region and the expressed variants are screened for the optimal combination of desired activity.

20 There are two principal variables in the construction of amino acid sequence variants; the location of the mutation site and the nature of the mutation. These may represent naturally occurring alleles or predetermined mutant forms made by mutating the DNA either to arrive at an allele or a variant not found in nature. In general, the location and nature of the
25 mutation chosen will depend upon the characteristic to be modified.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably about 1 to 10 residues and typically about 1 to 5 contiguous residues.

Amino acid sequence insertions include amino and/or
30 carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Other insertional variants include the fusion of the N- or C-terminus of the proteins to an immunogenic polypeptide e.g. bacterial polypeptides such as betalactamase or an enzyme
35 encoded by the *E. coli trp* locus, or yeast protein, bovine serum albumin, and chemotactic polypeptides. C-terminal fusions with proteins having a

long half-life such as immunoglobulin constant regions (or other immunoglobulin regions), albumin, or ferritin, are included.

Another group of variants are amino acid substitution variants. These variants have at least one amino acid residue in the protein molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include sites identified as the active site(s). Other sites of interest are those in which particular residues obtained from various species are identical. These positions may be important for biological activity. These sites, especially those falling within a sequence of at least three other identically conserved sites, are substituted in a relatively conservative manner. Such conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 1, or as further described below in reference to amino acid classes, are introduced and the products screened.

TABLE 1

| Original Residue | Exemplary Substitutions | Preferred Substitutions |
|-------------------------|--|--------------------------------|
| Ala (A) | val; leu; ile | val |
| Arg (R) | lys; gln; asn | lys |
| Asn (N) | gln; his; lys; arg | gln |
| Asp (D) | glu | glu |
| Cys (C) | ser | ser |
| Gln (Q) | asn | asn |
| Glu (E) | asp | asp |
| Gly (G) | pro | pro |
| His (H) | asn; gln; lys; arg | arg |
| Ile (I) | leu; val; met; ala; phe norleucine | leu |
| Leu (L) | norleucine, ile; val; met; ala; phe | ile |

| Original Residue | Exemplary Substitutions | Preferred Substitutions |
|------------------|--|-------------------------|
| Lys (K) | arg; gln; asn | arg |
| Met (M) | leu; phe; ile; | leu |
| Phe (F) | leu; val; ile; ala | leu |
| Pro (P) | gly | gly |
| Ser (S) | thr | thr |
| Thr (T) | ser | ser |
| Trp (W) | tyr | tyr |
| Tyr (Y) | trp; phe; thr; ser | phe |
| Val (V) | ile; leu; met; phe; ala; norleucine | leu |

Mutants, Variants and Homology - Proteins

5 Mutant polypeptides will possess one or more mutations which are deletions, insertions, or substitutions of amino acid residues. Mutants can be either naturally occurring (that is to say, purified or isolated from a natural source) or synthetic (for example, by performing site-directed mutagenesis on the encoding DNA). It is thus apparent that polypeptides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

10 An allelic variant will be a variant that is naturally occurring within an individual organism.

Protein sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the protein will be the equivalent protein which occurs naturally in another species.

15 Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the protein. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same

20 Class and even more preferably the same Order.

A protein at least 50% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) *Ad. Appl Math.*, 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) *J. Mol. Biol.*, 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 70% or 80% and more preferably at least 90% identical to the protein of the present invention. This will generally be over a region of at least 20, preferably at least 30, contiguous amino acids.

10 Mutants, Variants and Homology - Nucleic Acids

Mutant polynucleotides will possess one or more mutations which are deletions, insertions, or substitutions of nucleotide residues. Mutants can be either naturally occurring (that is to say, isolated from a natural source) or synthetic (for example, by performing site-directed mutagenesis on the DNA). It is thus apparent that polynucleotides of the invention can be either naturally occurring or recombinant (that is to say prepared using recombinant DNA techniques).

An allelic variant will be a variant that is naturally occurring within an individual organism.

Nucleotide sequences are homologous if they are related by divergence from a common ancestor. Consequently, a species homologue of the polynucleotide will be the equivalent polynucleotide which occurs naturally in another species. Within any one species a homologue may exist as numerous allelic variants, and these will be considered homologues of the polynucleotide. Allelic variants and species homologues can be obtained by following standard techniques known to those skilled in the art. Preferred species homologues include those obtained from representatives of the same Phylum, more preferably the same Class and even more preferably the same Order.

A polynucleotide at least 70% identical, as determined by methods well known to those skilled in the art (for example, the method described by Smith, T.F. and Waterman, M.S. (1981) *Ad. Appl Math.*, 2: 482-489, or Needleman, S.B. and Wunsch, C.D. (1970) *J. Mol. Biol.*, 48: 443-453), to that of the present invention are included in the invention, as are proteins at least 80% or 90% and more preferably at least 95% identical to the

polynucleotide of the present invention. This will generally be over a region of at least 60, preferably at least 90, contiguous nucleotide residues.

Antibody Production

5 The term "antibody" should be construed as covering any specific binding substance having a binding domain with the required specificity. Thus, the term covers antibody fragments, derivatives, functional equivalents and homologues of antibodies, including any polypeptide including an immunoglobulin binding domain, whether natural or synthetic. Chimaeric
10 molecules including an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included.

Antibodies, either polyclonal or monoclonal, which are specific for a protein of the present invention can be produced by a person skilled in the art using standard techniques such as, but not limited to, those described by
15 Harlow et al. *Antibodies: A Laboratory Manual*, Cold Springs Harbor Laboratory Press (1988), and D. Catty (editor), *Antibodies: A Practical Approach*, IRL Press (1988).

Various procedures known in the art may be used for the production of polyclonal antibodies to epitopes of a protein. For the production of
20 polyclonal antibodies, a number of host animals are acceptable for the generation of antibodies by immunization with one or more injections of a polypeptide preparation, including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response in the host animal, depending on the host species, including but not limited to
25 Freund's (complete and incomplete), mineral gels such as aluminium hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

30 A monoclonal antibody to an epitope of a protein may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, *Nature* 256, 493-497), and the more recent human B-cell
35 hybridoma technique (Kesber *et al.* 1983, *Immunology Today* 4:72) and EBV-hybridoma technique (Cole *et al.* 1985, *Monoclonal Antibodies and*

Cancer Therapy, Alan R. Liss, Inc. pp. 77-96). In addition, techniques developed for the production of "chimeric antibodies" by splicing the genes from an antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity may be used (Morrison *et al.* 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger *et al.* 1984 Nature 312:604-608; Takeda *et al.* 1985 Nature 31:452-454). Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce 4-specific single chain antibodies.

Recombinant human or humanized versions of monoclonal antibodies are a preferred embodiment for human therapeutic applications. Humanized antibodies may be prepared according to procedures in the literature (e.g. Jones *et al.* 1986, Nature 321:522-25; Reichman *et al.* 1988, Nature 332:323-27; Verhoeyen *et al.* 1988, Science 239:1534-36). The recently described "gene conversion mutagenesis" strategy for the production of humanized monoclonal antibody may also be employed in the production of humanized antibodies (Carter *et al.* 1992 Proc. Natl. Acad. Sci. U.S.A. 89:4285-89). Alternatively, techniques for generating the recombinant phage library of random combinations of heavy and light regions may be used to prepare recombinant antibodies (e.g. Huse *et al.* 1989 Science 246:1275-81).

Antibody fragments which contain the idiotype of the molecule such as $F(ab^1)$ and $F(ab^2)$ may be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab)$ E2 fragment which can be produced by pepsin digestion of the intact antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the two Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent. Alternatively, Fab expression libraries may be constructed (Huse *et al.* 1989, Science 246:1275-1281) to allow rapid and easy cloning of a monoclonal Fab fragment with the desired specificity to a protein.

Adjuvants and Carriers

Pharmaceutically acceptable carriers or diluents include those used in compositions suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. They are

non-toxic to recipients at the dosages and concentrations employed.

Representative examples of pharmaceutically acceptable carriers or diluents include, but are not limited to water, isotonic solutions which are preferably buffered at a physiological pH (such as phosphate-buffered saline or

5 Tris-buffered saline) and can also contain one or more of, mannitol, lactose, trehalose, dextrose, glycerol, ethanol or polypeptides (such as human serum albumin). The compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy.

10 As mentioned above the composition may include an adjuvant. As will be understood an "adjuvant" means a composition comprised of one or more substances that enhances the immunogenicity and efficacy of a vaccine composition. Non-limiting examples of suitable adjuvants include squalane and squalene (or other oils of animal origin); block copolymers; detergents
15 such as Tween®-80; Quil® A, mineral oils such as Drakeol or Marcol, vegetable oils such as peanut oil; *Corynebacterium*-derived adjuvants such as *Corynebacterium parvum*; *Propionibacterium*-derived adjuvants such as *Propionibacterium acne*; *Mycobacterium bovis* (Bacille Calmette and Guerin or BCG); interleukins such as interleukin 2 and interleukin 12; monokines
20 such as interleukin 1; tumour necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminium hydroxide or Quil-A aluminium hydroxide; liposomes; ISCOM adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as muramyl dipeptides or other derivatives; Avridine; Lipid A derivatives; dextran sulfate; DEAE-Dextran or
25 with aluminium phosphate; carboxypolymethylene such as Carbopol' EMA; acrylic copolymer emulsions such as Neocryl A640 (e.g. U.S. Pat. No. 5,047,238); vaccinia or animal poxvirus proteins; sub-viral particle adjuvants such as cholera toxin, or mixtures thereof.

30 Gene/DNA Isolation

The DNA encoding a protein may be obtained from any cDNA library prepared from tissue believed to express the gene mRNA and to express it at a detectable level. DNA can also be obtained from a genomic library.

35 Libraries are screened with probes or analytical tools designed to identify the gene of interest or the protein encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal

triesters, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding residues for each amino acid residue.

Substantially Purified

By "substantially purified" we mean a polypeptide that has been separated from lipids, nucleic acids, other polypeptides, and other contaminating molecules.

Hybridisation

The polynucleotide sequence of the present invention may hybridise to the respective sequence set out SEQ. ID. NOS. 2, 4, or 6 under high stringency. As used herein, stringent conditions are those that (i) employ low ionic strength and high temperature for washing after hybridization, for example, 0.1 x SSC and 0.1% (w/v) SDS at 50°C; (ii) employ during hybridization conditions such that the hybridization temperature is 25°C lower than the duplex melting temperature of the hybridizing polynucleotides, for example 1.5 x SSPE, 10% (w/v) polyethylene glycol 6000, 7% (w/v) SDS, 0.25 mg/ml fragmented herring sperm DNA at 65°C; or (iii) for example, 0.5M sodium phosphate, pH 7.2, 5mM EDTA, 7% (w/v) SDS and 0.5% (w/v) BLOTTO at 70°C; or (iv) employ during hybridization a denaturing agent such as formamide, for example, 50% (v/v) formamide with 5 x SSC, 50mM sodium phosphate (pH 6.5) and 5 x Denhardt's solution (32) at 42°C; or (v) employ, for example, 50% (v/v) formamide, 5 x SSC, 50mM sodium phosphate (pH 6.8), 0.1% (w/v) sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50µg/ml) and 10% dextran sulphate at 42°C.

EXAMPLE 1

This example describes the cloning and characterisation of a protease from *Moraxella bovis*.

5

Bacteria and construction of a genomic library

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6). *Escherichia coli* strain DH5 α has been previously described (7, 8).

10

All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted.

General cloning and DNA techniques were as described (9) unless otherwise noted.

A genomic library was constructed by carrying out partial *Sau*3A digests on genomic DNA extracted from *M. bovis* strain Dalton 2d using a CTAB method which is outlined below. This DNA was size fractionated using a NaCl gradient (10) and ligated with the cosmid cloning vector pHC79 (11) which had been previously digested with *Bam*HI. This DNA was packaged into lambda bacteriophage heads using the Packagene Lambda DNA packaging system (Promega, Madison, WI, USA) and this was used to transduce the *E. coli* strain DH5 α . The library was stored in 96 well trays (50% glycerol / luria broth / ampicillin (50 μ g/ml)) at -70°C.

25

CTAB genomic DNA extraction from *M. bovis*

A 5ml brain heart infusion (BHI) (Oxoid Ltd., Basingstoke, Hampshire, U.K.) broth was inoculated with a colony of Dalton 2d taken from a fresh overnight culture on horse blood agar and incubated with shaking at 37°C for 6 hours. This culture was used to inoculate 50ml of BHI broth which was grown with shaking at 37°C overnight. 40ml of the culture was transferred to an SS34 tube and the cells pelleted at 3000 \times g for 10 minutes. Following resuspension of the pellet in 9.5ml of 25% sucrose in TE buffer (10mM Tris, 1mM EDTA (pH8)), 500 μ l of 10% SDS, 50 μ l of 20mg/ml proteinase K and 20 μ l of 10mg/ml RnaseA were added and this mixture incubated in an orbital shaker for 1 hour at 37°C. To this mixture, 1.8ml of 5M NaCl and 1.5ml of a CTAB (N-Cetyl-N,N,N-trimethyl-ammonium bromide) / NaCl solution was added and incubation continued for 20 minutes at 65°C. The DNA was

30

35

extracted using phenol/chloroform and precipitated with 0.6 volumes of isopropanol. The resulting DNA was washed in 70% ethanol, dried and resuspended in 2ml of TE buffer.

5 **Screening of genomic library for enzyme activity**

The genomic library was cultured on skim milk agar to screen for the presence of a clone displaying protease activity (double strength Columbia agar base (Oxoid Ltd., Basingstoke, Hampshire, U.K.) / 10% skim milk) for 24 hours at 37°C followed by refrigeration at 4°C for one to two days.

10 A single clone from the genomic library was detected as having activity against skim milk agar. DNA analysis confirmed that the clone contained a fragment of *M. bovis* Dalton 2d genomic DNA approximately 40 kilobases in size. The construct was designated pJF1.

15 **Nucleotide sequence of the *M. bovis* protease clone pJF1**

Plasmid and cosmid DNA for automated sequencing was extracted using the Wizard Plus SV Minipreps DNA Purification System (Promega, Madison, WI, USA) and the Qiagen Plasmid Midi Kit (Qiagen Pty. Ltd., Clifton Hill, Vic, Australia), respectively.

20 The nucleotide sequence of the insert DNA was determined using the process of "primer walking" (12). This was achieved using synthetic oligonucleotides (Bresatec / Geneworks, Thebarton, SA, Australia) and the dye terminator cycle sequencing ready reaction (Perkin Elmer Corporation, Norwalk, CT, USA). The resulting sequence was analysed on an Applied
25 Biosystems 373A DNA sequencer.

Automated sequencing revealed an open reading frame of 3345bp capable of encoding a protein of 1115 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 1 together with the corresponding amino acid sequence which is predicted to encode a protein
30 with a molecular weight of 120kDa. The amino acid sequence is shown in SEQ. ID. NO. 1 and the DNA sequence is shown in SEQ. ID. NO. 2.

The putative start codon for the mature protease protein was identified by the presence of a possible ribosome binding site upstream. This RBS was identified by its similarity to the consensus sequence for the *E. coli* RBS and
35 that previously identified for the *M. bovis* pilin genes (AGGAG) (27)

Due to the secreted nature of the protease, it was assumed that it would contain in its N-terminal sequence a signal peptide which would be used in the secretion of the protein. This analysis was carried out using a prediction program (SignalP) available through the Expasy website (http://www.expasy.ch/tools/), which allows for the identification of prokaryotic signal peptides and predicts possible cleavage sites. This analysis only identified a signal peptide using the start codon indicated in the accompanying protein/DNA sequence.

10 Sequence comparisons

Comparisons of the deduced amino acid sequence with those in the database were carried out using the BlastX and BlastP programs (13) which are available at <http://www.ncbi.nlm.nih.gov>.

At the amino acid level, the protease cloned from Dalton 2d displayed the following similarity and identity to the proteins listed.

| Organism | Protein | Similarity | Identity |
|--------------------------------|--|------------|----------|
| <i>Serratia marcescens</i> | ssp-h2 - serine protease autotransporter | 39% | 23% |
| <i>Serratia marcescens</i> | ssp-h1 - serine protease autotransporter | 37% | 22% |
| <i>Pseudomonas fluorescens</i> | serine protease homologue | 34% | 20% |
| <i>Pseudomonas tolaasii</i> | serine protease | 35% | 21% |

More generally the 5' domain of the *M. bovis* protease displays homology to a family of subtilisins (serine proteases) while the 3' region resembles a number of outer membrane proteins.

The *M. bovis* sequence was found to contain a highly proline rich region which distinguished it from all other proteins to which it was closely related.

25 Protease type encoded by pJF1

In order to identify the type of protease activity encoded by pJF1, a range of specific protease inhibitors were examined for their effect on the expression of the *M. bovis* protease.

Results showed that the protease gene cloned in pJF1 is present in all strains of *M. bovis* examined.

EXAMPLE 2

This example describes the cloning and characterisation of a lipase from *Moraxella bovis*.

5

Bacteria and construction of a plasmid library

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6). *Escherichia coli* strain MC1061 has been previously described (16).

10 All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted. General cloning and DNA techniques were as described (9) unless otherwise noted.

A plasmid library was constructed in the cloning vector pBR322 (17). This was done by partially digesting genomic DNA extracted from Dalton 2d (using the CTAR method described in Example 1) with *Sau*3A under
15 conditions that maximised the amount of DNA in the range of 1 to 2kb. This DNA was ligated with pBR322 which had been previously digested with *Bam*HI. The ligated DNA was electroporated (2.5kV, 200Ω and 200μF, for a theoretical time constant of 4.7) into electrocompetent *E. coli* MC1061 cells.

20

Screening of plasmid library for lipase expression

Following electroporation of the ligated DNA into MC1061 cells, recombinant clones displaying lipase activity were detected by culturing the library for 24 hours at 37°C on media containing Tween 80 [10ml Tween 80
25 (Sigma, St Louis, MO, USA), 5g NaCl, 3g agar No.1 (Oxoid Ltd., Basingstoke, Hampshire, U.K.), 10g peptone, 0.1g CaCl₂·H₂O / litre].

Twenty eight out of 24,000 clones screened were found to be displaying lipase activity. DNA analysis confirmed that all of these clones contained one 5.4kb fragment of DNA in common. One clone was chosen to
30 continue work with and this was designated pMB1.

In some experiments (below), a photometric assay of extracellular lipase activity was performed with p-nitrophenylpalmitate as the substrate (18, 19). Strains of *E. coli* and/or *M. bovis* were grown at 37°C for the required time points. Cell free culture supernatant (100μl) was mixed with
35 2.4ml of enzyme buffer (19) to assay secreted lipase activity. After 30 minutes incubation at 37°C, the optical density at 410nm was determined.

One enzyme unit was defined as the amount of enzyme that releases 1 nmol of p-nitrophenyl from p-nitrophenylpalmitate $\text{ml}^{-1} \text{min}^{-1}$. Under the conditions described by Stuer *et al.*, (18), an optical density at 410nm of 0.041 is equivalent to 1 enzyme unit.

5

Nucleotide sequence of the *M. bovis* lipase clone pMB1

Plasmid pMB1 was subjected to automated DNA sequencing using the methodology described in Example 1.

10 This analysis revealed an open reading frame of 1851bp capable of encoding 617 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 2 together with the corresponding amino acid sequence that is predicted to encode a protein with a molecular weight of 65.8kDa. The amino acid sequence is shown in SEQ. ID. NO. 3 and the DNA sequence is shown in SEQ. ID. NO. 4.

15 The techniques set out above in respect of the protease were used to identify the potential start codon for the lipase protein.

Sequence comparisons

20 Sequence comparisons were made using the methodology described in Example 1.

At the amino acid level, the lipase cloned from *M. bovis* Dalton 2d was shown to display the following similarity and identity to the proteins listed.

| Organism | Protein | Similarity | Identity |
|--------------------------------|-------------------------|------------|----------|
| <i>Xenorhabdus luminescens</i> | triacylglycerol lipase | 36% | 24% |
| <i>Pseudomonas putida</i> | hypothetical protein | 36% | 24% |
| <i>Salmonella typhimurium</i> | outer membrane esterase | 35% | 23% |
| <i>Pseudomonas aeruginosa</i> | lipase / esterase | 36% | 23% |

25 The *M. bovis* lipase was identified as being a possible new member of the GDSL family (20) of lipolytic enzymes.

N-terminal sequencing carried out on the lipase mature protein

The required strains of *E. coli* were cultured overnight with shaking at 37°C in 500mls of luria broth. The cells were pelleted at 5,000 rpm for 15 mins and the supernatant filtered through a 0.45µm filter. Solid ammonium sulfate was added to the supernatant to 60% saturation (180g / 500ml), and dissolved at 4°C with stirring for 30 minutes. This mixture was left at 4°C overnight and the precipitated proteins pelleted at 7,000 rpm for 30 mins. The proteins were resuspended in 3ml of double distilled water and the solubilised proteins dialysed against double distilled water overnight to remove any salt. The resulting mixture was filtered through a 0.45µm filter and stored at -20°C.

Following separation of the proteins by SDS-PAGE, the proteins were transferred to PVDF membrane and excised. The protein was subjected to automated (Edman degradation) sequence analysis (28) with vapour phase delivery of critical reagents (29) in an automated sequenator (model 470A; Applied Biosystems) (Applied Biosystems Division, Foster City, CA, USA) in conjunction with a PTH amino acid separation system (model 120A PTH analyzer; Applied Biosystems).

Using this technique 17 amino acids with two gaps were identified
K E F S Q V I I F G D S L X D X G (SEQ ID NO:7)
which corresponds exactly with amino acids 26 through to 42 shown on the accompanying sequence. This result also indicated that the protein most likely includes an amino terminal signal peptide which is involved in the secretion of the protein. This amino terminal corresponds to amino acids 1 through to 25 in the accompanying sequence.

25 **Raising antibodies to the lipase in rabbits**

Antibody to the recombinant lipase was raised in rabbits by injecting ammonium sulfate precipitated supernatant from *E. coli* MC1061/pMB4. Prior to vaccination, the lipase preparation was inactivated by heating to 90°C for 90min. 30µg of this protein was injected at 2 weekly intervals for 4 weeks. The primary inoculum was emulsified with Freund's complete adjuvant and subsequent vaccinations with Freund's incomplete adjuvant.

35 **Heat stability of *M. bovis* lipase**

The recombinant lipase cloned from *M. bovis* Dalton 2d was found to be very heat stable since it required heating at 90°C for 105 minutes for the

activity to be reduced by 97%. Figure 3 illustrates this phenomenon with enzyme activity expressed as "lipase enzyme units" as determined in the extracellular lipase assay.

5 **Relative expression levels of native versus recombinant lipase**

An experiment was performed to plot growth rate with lipase production and to compare production of the recombinant lipase from MC1061/pMB1 with that of the native form of the lipase from *M. bovis* Dalton 2d. Figure 4 illustrates that the two strains grow at approximately the same rate but they do not reach the same cell density, with Dalton 2d substantially lower after 9 hours than MC1061/pMB1. Lipase expression levels were greatest from the pMB1 construct in *E. coli* compared to native lipase expression from *M. bovis* Dalton 2d.

This result was further substantiated when proteins from cell-free supernatants of either the *E. coli* clone or *M. bovis* Dalton 2d were ammonium sulfate precipitated and analysed by SDS-PAGE and western blot using antisera to the recombinant heat-deactivated lipase.

Ammonium sulfate precipitated supernatants were prepared from overnight cultures of *E. coli* or *M. bovis* that had been shaken at 37°C in either 500mls of Luria broth or brain heart infusion broth, respectively. Cells were pelleted at 5000 × g for 15 minutes and the supernatant filtered through a 0.45µm filter. Solid ammonium sulfate was added to the supernatant to 60% saturation (180g / 500ml) and dissolved at 4°C with stirring for 30 minutes. This mixture was left at 4°C overnight and the precipitated protein pelleted at 7000 × g for 30 minutes. Proteins were resuspended in 3ml of double distilled water and the solubilised proteins dialysed against double distilled water overnight to remove any salt. The resulting mixture was filtered through a 0.45µm filter and stored at -20°C.

Protein samples (100µl) were prepared for SDS-PAGE by resuspension in 100µl of 2x sample buffer (5ml 0.5M Tris (pH6.8), 8ml 10% SDS, 4ml glycerol, 0.8ml β-mercaptoethanol, 1ml double distilled H₂O, bromophenol blue) and heating to 100°C for 5 minutes. The proteins were separated on a 12.5% polyacrylamide gel using the buffer system of Laemmli (21).

Western blots were carried out according to the method of Towbin *et al.*, (22) and following separation of proteins by SDS-PAGE and transfer to nitrocellulose using the Bio-Rad minicell (Bio-Rad, Hercules, CA, USA)

transfer system. Filters were immunoblotted with the recombinant lipase antiserum (at a concentration of 1/100) which had been adsorbed against MC1061 cells. The antiserum was raised against ammonium sulfate precipitated recombinant lipase which had been heat deactivated (1 hour 45 minutes at 90°C) and used to inoculate rabbits (three doses of 50µg each) at 4 week intervals. Blood samples were collected from the marginal ear vein prior to immunisation and at each vaccination time point.

The results showed a prominent band present in the recombinant lipase positive construct MC1061/pMB1 that is detectable in relatively minor amounts in *M. bovis* Dalton 2d preparation. The protein detected with the antisera was approximately the same size as that of the predicted molecular weight for the *M. bovis* lipase (65.8kDa).

Lipase type encoded by pMB1

Thin layer chromatography (TLC) was used to determine whether the lipase of *M. bovis* Dalton 2d displayed phospholipase activity.

Characterisation of phospholipase type essentially followed a previously described method (23) except that the results of separation on Silica Gel 60 plates were visualised by developing with a 10% ethanolic solution of molybdophosphoric acid at 100°C. All reagents used were purchased from Sigma (Sigma, St Louis, MO, USA).

TLC determined that the *M. bovis* lipase displayed the same enzyme specificity as that of a commercially-available phospholipase B when lysophosphatidylcholine and phosphatidylcholine were used as substrates (data not shown).

Conservation of lipase among *M. bovis*

A southern blot using an internal fragment of the Dalton 2d lipase coding region was used to investigate whether the lipase gene was present in strains of *M. bovis* representing the known pilus serotypes.

Genomic DNA extracted from the strains of *M. bovis* representing each of the known pilus serotypes (15) was digested with *Hind*III and separated using agarose gel electrophoresis. The DNA was transferred to a Hybond N+ filter (Amersham, Little Chalfont, Buckinghamshire, U.K.) using a previously described method (9). The probe used in the southern hybridisation was a *Hind*III fragment that contained sequence internal to the lipase coding

region. This fragment was labelled with $\alpha^{32}\text{P}$ -dATP using the Megaprime labelling system (Amersham, Little Chalfont, Buckinghamshire, U.K.) according to the manufacturers instructions. High stringency conditions were used (hybridisation temperature 68°C; 2 washes at room temperature in 2 × SSC / 0.1% SDS; 1 wash at 68°C in 0.1 × SSC / 0.1% SDS) and the resulting filters were exposed to autoradiographic film (Kodak, Rochester, New York, USA) for 5 to 24 hours before developing.

Results showed that the lipase gene is present in all strains of *M. bovis* examined.

To confirm whether or not the lipase gene was expressed in each of the serotype representative strains, antisera raised against recombinant heat deactivated lipase was used in a western blot analysis of whole cell preparations. Results showed that the lipase was indeed being expressed by all of these *M. bovis* strains.

EXAMPLE 3

Bacteria and construction of a haemolysin clone

Moraxella bovis strain Dalton 2d was a field isolate collected from a bovine eye and characterised by CSIRO Animal Health, Parkville, Australia (6).

All of the *M. bovis* strains representative of the known pilus serotypes express a haemolytic activity that is detected on horse blood agar.

Escherichia coli strain degP4::Tn5 has a leaky outer membrane and is defective in proteolysis and has been previously described (24).

All enzymes were purchased from Promega (Madison, WI, USA) except where otherwise noted.

General cloning and DNA techniques were as described (9) unless otherwise noted.

A *phoA* fusion technique that allows for the identification of exported proteins (25) was utilised with some modifications. Genomic DNA from *M. bovis* Dalton 2d (prepared using the CTAB method described in Example 1) was partially digested with *Sau*3A. Restricted DNA was ligated with a series of vectors that allow fusions with an alkaline phosphatase gene in three different reading frames. The ligated DNA was electroporated into *E. coli* degP4::Tn5 and the resulting clones screened on Luria agar containing

ampicillin (50µg/ml) and X-P (200µg/ml) (5-bromo-3-chloro-indolyl phosphate). Selection of clones relies on the observation that if the fragment is cloned in frame and contains an export sequence the resulting colony will be blue in colour. The leaky *E. coli* strain allows the outer membrane-bound proteins and secreted proteins (both fused with *phoA*) to be distinguished from non-secreted fusion proteins.

Sequencing of the *M. bovis* haemolysin determinant

Clones selected for the presence of a secreted or outer membrane protein gene sequence were subjected to automated DNA sequencing using the methods described in Example 1. One of these clones, pMbh1, was found to contain 200bp of DNA which displayed high homology to the A subunit of other RTX toxins. Inverse PCR and degenerate oligonucleotides were utilised to obtain the sequence of the entire A subunit. The open reading frame of 2784bp was capable of encoding 928 amino acids. The sequence is written in the 5' to 3' direction and is shown in Figure 5 together with the corresponding amino acid sequence that is predicted to encode a protein with a molecular weight of 98.8kDa. The amino acid sequence is shown in SEQ. ID. NO. 5 and the DNA sequence is shown in SEQ. ID. NO. 6.

The putative start codon was identified using the RBS technique outlined above. A signal peptide analysis was not carried out as the A subunit is not secreted on its own. However as the protein sequence of these proteins (RTX) is quite highly conserved, on amino acid homologies alone this start codon was the one of choice.

25

Sequence homology

At the amino acid level the *M. bovis* Dalton 2d haemolysin gene product shows striking similarity to the A subunit of the of several RTX and other haemolysins as shown in the following table.

Genomic DNA extracted from the seven serotype strains of *M. bovis* (15) was digested with *EcoRV* and separated using agarose gel electrophoresis. The DNA was transferred to a Hybond N+ filter (Amersham,

Little Chalfont, Buckinghamshire, U.K.) using a previously described method (9). The probe used was a PCR amplified product that contained all of the coding region from the A subunit of the RTX haemolysin of *M. bovis*. This fragment was labelled with $\alpha^{32}\text{P}$ -dATP using the Megaprime labelling system (Amersham, Little Chalfont, Buckinghamshire, U.K.) according to the manufacturers instructions. High stringency conditions were used (hybridisation temperature 68°C; 2 washes at room temperature in $2 \times \text{SSC} / 0.1\% \text{SDS}$; 1 wash at 68°C in $0.1 \times \text{SSC} / 0.1\% \text{SDS}$) and the resulting filters were exposed to autoradiographic film (Kodak, Rochester, New York, USA) for 5 to 24 hours before developing.

Results showed that the gene encoding the RTX A haemolysin subunit was conserved in all seven representative strains of *M. bovis* examined. Interestingly, each of these strains is known to display the haemolytic phenotype on horse blood agar. In contrast, the non-haemolytic *M. bovis* strain Gordon 26L3 did not hybridise to the RTX A gene probe possibly suggesting that *M. bovis* contains only a single structural gene responsible for the haemolytic phenotype detected on horse blood agar.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

References

1. Moore L. J., and A. W. D. Lepper. (1991). *Vet Microbiol.* 29:75-83.
2. Lehr, C. H., G. Jayappa and R. A. Goodnow. (1985). *Cornell Vet.* 75:484-
5 492.
3. Pugh, G. W., D. E. Hughes and G. D. Booth. (1977). *Am J Vet Res.* 38:1519-1522.
4. Frank, S. K., and J. D. Gerber. (1981). *J Clin Microbiol.* 13(2):269-271.
5. Billson, F.M., J. L. Hodgson, A. W. Lepper, W. P. Michalski, C. L.
10 Schwartzkoff, P. R. Lehrbach, and J. M. Tennent. (1994). *FEMS Microbiol Lett.* 124(1):69-73.
6. Elleman, T. C., P. A. Hoyne, and A. W. D. Lepper. (1990). *Infect Immun.* 58(6):1678-1684.
7. Woodcock, D. M., P. J. Crowther, J. Doherty, S. Jefferson, E. DeCruz, M.
15 Noyer-Weidner, S. S. Smith, M. Z. Michael, and M. W. Graham. (1989). *Nucleic Acids Res.* 17(9):3469-3478.
8. Raleigh, E. A., K. Lech, and R. Brent. (1989). In *Current Protocols in Molecular Biology* eds. Ausubel, F. M. et al., Publishing Associates and Wiley Interscience; New York. Unit 1.4.
- 20 9. Sambrook, J., E. F. Fritsch, and T. Maniatis. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd edition. Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York.
10. Dillela, A. G., and S. L. C. Woo. (1985). *Focus.* 7(2):1-5.
11. Hohn, B., and J. Collins. (1980). *Gene.* 11(3-4):291-298.
- 25 12. Narberhaus, F., K. Giebel, and H. Bahl. (1992). *J Bacteriol.* 174(10):3290-3299.
13. Altschul, S. F., T. L. Madden, A. A. Schaffer, Z. Jinghui, Z. Zhang, W. Miller, and D. J. Lipman. (1997). *Nucleic Acids Res.* 25:3389-3402.
14. Bourgeau, G., H. Lapointe, P. Peloquin, and D. Mayrand. (1992).
30 *Infection and Immunity.* 60(8):3186-3192.
15. Ausubel, F. M. R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl. (1994). *Current Protocols in Molecular Biology*. Green Publishing Associates, Inc. and John Wiley and Sons Inc. New York.
16. Wertman, K. F., A. R. Wyman, and D. Botstein. (1986). *Gene.* 49(2):253-
35 262.

17. Bolivar F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heynecker, and H. W. Boyer. (1977). *Gene*. 2(2):95-113.
18. Stuer, W., K. E. Jaeger, and U. K. Winkler. (1986). *J Bacteriol*. 168:1070-1074.
- 5 19. Winkler, U. K., and M. Stuckman. (1979). *J Bacteriol*. 138:663-670.
20. Upton, C., and J. T. Buckley. (1995). *Trends Biochem Sci*. 20(5):178-9.
21. Laemmli, U. K. (1970). *Nature*. 227(259):680-685.
22. Towbin, H., T. Staehlin, and J. Gordon. (1979). *Proc Natl Acad Sci USA*. 76(9):4350-4354.
- 10 23. Fifis, T., C. Costopoulos, and J. A. Vaughn. (1996). *Vet Microbiol*. 49:219-233.
24. Strauch, K. L., and J. Beckwith. (1988). *Proc Natl Acad Sci USA*. 85(5):1576-1580.
25. Gilaldi, M., C. I. Champion, D. A. Haake, D. R. Blanco, J. F. Miller, J. N. Miller, and M. A. Lovett. (1993). *J Bacteriol*. 175:4129-4136.
- 15 26. Mackman, N., J-M. Nicaud, L. Gray, and I. B. Holland. (1985). *Mol Genet*. 201:282-288.
27. Atwell, J. L., J. M. Tennent, A. W. Lepper and T. C. Elleman. (1994). *J Bacteriol* 176(16):4875-82 .
- 20 28. Edman, P., and C. Begg. (1967). *Eur. J. Biochem*. 1:80-91.
29. Hewick, R. M., M. W. Hunkapillar, L. E. Hood, and W. J. Dreyer. 1981. *J Biol Chem*. 256: 7990-7997

Claims:

1. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO. 1 from amino acid 37 to 1114, or a sequence having at least 50% identity thereto, or a functional fragment thereof.
2. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.
3. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.
4. A polypeptide as claimed in claim 1, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:1 from amino acid 37 to 1114.
5. A polypeptide as claimed in any one of claims 1 to 4, the polypeptide having protease activity.
6. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide as claimed in any one of claims 1 to 5.
7. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:2 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.
8. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 70% identity with the sequence shown in SEQ. ID. NO:2.
9. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 80% identity with the sequence shown in SEQ. ID. NO:2.

10. A nucleic acid molecule as claimed in claim 7, the nucleic acid molecule comprising a sequence having least 90% identity with the sequence shown in SEQ. ID. NO:2.
- 5 11. A composition for use in raising an immune response in an animal, the composition comprising the polypeptide as claimed in any one of claims 1 to 5 or a nucleic acid sequence as claimed in claim 6 and optionally a carrier and/or adjuvant.
- 10 12. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO:3 from amino acid 26 to 616, or a sequence having at least 50% identity thereto, or a functional fragment thereof.
- 15 13. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.
- 20 14. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.
- 25 15. A polypeptide as claimed in claim 12, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:3 from amino acid 26 to 616.
- 30 16. A polypeptide as claimed in any one of claims 12 to 15, the polypeptide having lipase activity.
- 35 17. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide of any one of claims 12 to 16.
18. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:4 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

19. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 70% identity with the sequence shown in SEQ. ID. NO:4.
- 5 20. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 80% identity with the sequence shown in SEQ. ID. NO:4.
21. A nucleic acid molecule as claimed in claim 18, the nucleic acid molecule comprising a sequence having at least 90% identity with the sequence shown in SEQ. ID. NO:4.
- 10 22. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide as claimed in any one of claims 12 to 16 or a nucleic acid sequence as claimed in claim 17 and optionally a carrier and/or adjuvant.
23. A polypeptide, the polypeptide having an amino acid sequence as set out in SEQ. ID. NO:5, or a sequence having at least 60% identity thereto, or a functional fragment thereof.
- 20 24. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 70% identity with the sequence shown in SEQ. ID. NO:5.
- 25 25. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 80% identity with the sequence shown in SEQ. ID. NO:5.
26. A polypeptide as claimed in claim 23, the polypeptide having a sequence of at least 90% identity with the sequence shown in SEQ. ID. NO:5.
- 30 27. A polypeptide as claimed in any one of claims 23 to 26, the polypeptide having haemolysin activity.
28. A nucleic acid molecule, the nucleic acid molecule comprising a sequence encoding a polypeptide of any one of claims 23 to 27.
- 35

29. A nucleic acid molecule comprising a sequence as set out in SEQ. ID. NO:6 or a sequence having at least 60% identity thereto, or a sequence which hybridises thereto under stringent conditions.

5 30. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 70 % identity with the sequence shown in SEQ. ID. NO:6.

10 31. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 80 % identity with the sequence shown in SEQ. ID. NO:6.

15 32. A nucleic acid molecule as claimed in claim 29, the nucleic acid molecule comprising a sequence having at least 90 % identity with the sequence shown in SEQ. ID. NO:6.

20 33. A composition for use in raising an immune response in an animal, the composition comprising a polypeptide of any one of claims 23 to 27 or a nucleic acid sequence of claim 28 and optionally a carrier and/or adjuvant.

25 34. A composition for use in raising an immune response in an animal directed against *Moraxella*, the composition comprising at least one polypeptide selected from the group consisting of a polypeptide as claimed in any one of claims 1 to 5, a polypeptide as claimed in any one of claims 12 to 16, and a polypeptide as claimed in any one of claims 23 to 27, and optionally including an adjuvant or carrier.

30 35. A composition as claimed in claim 34, the composition comprising a polypeptide as claimed in any one of claims 23 to 27 and either one of, or preferably both of, a polypeptide as claimed in any one of claims 1 to 5 and a polypeptide as claimed in any one of claims 12 to 16.

35 36. A composition as claimed in claim 34 or claim 35 wherein the *Moraxella* is *M. bovis* or *M. catarrhalis*.

37. A composition as claimed in claim 34 or claim 35 wherein the *Moraxella* is *M. bovis*.
38. An antibody raised against a polypeptide selected from the group
5 consisting of a polypeptide as claimed in any one of claims 1 to 5, a
polypeptide as claimed in any one of claims 12 to 16, and a polypeptide as
claimed in any one of claims 23 to 27.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
8 March 2001 (08.03.2001)

PCT

(10) International Publication Number
WO 01/16172 A1

(51) International Patent Classification⁷: **C07K 14/22**,
16/12, A61K 38/48, 39/095, C12N 15/31

Hawthorn, VIC 3122 (AU). **TENNENT, Jan** [AU/AU]; 6
Campbell Grove, Hawthorn East, VIC 3123 (AU).

(21) International Application Number: PCT/AU00/01048

(74) Agent: **F B RICE & CO**; 139 Rathdowne Street, Carlton,
VIC 3053 (AU).

(22) International Filing Date: 31 August 2000 (31.08.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PQ 2571 31 August 1999 (31.08.1999) AU

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(71) Applicants (*for all designated States except US*): **COM-
MONWEALTH SCIENTIFIC AND INDUSTRIAL
RESEARCH ORGANISATION** [AU/AU]; Limestone
Avenue, Campbell, ACT 2601 (AU). **THE UNIVERSITY
OF MELBOURNE** [AU/AU]; Royal Parade, Parkville,
VIC 3052 (AU).

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— With international search report.

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **FARN, Jacinta**
[AU/AU]; 61 Glass Street, Essendon, VIC 3040 (AU).
STRUGNELL, Richard [AU/AU]; 17 Salisbury Grove,

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: **VACCINE ANTIGENS OF MORAXELLA**

(57) Abstract: The present invention relates to antigens of *Moraxella*, in particular, *Moraxella bovis*, nucleic acid sequences encod-
ing these antigens and formulations for use in raising an immune response against *Moraxella*.

WO 01/16172 A1

1/7

1 ttctcatggt tgacagctta tcatcgataa gctttaatgc ggtagtttat cacagttaaa ttgctaacgc agtcaggcac cgtgtatgaa
 91 atctaaacaat gcgctcatcg tcatcctcgg caccgtcacc ctggatgctg taggcataag cttggttatg ccggtactgc cgggectctt
 181 gcgggatata gtccattccg acagcatcgc cagtcactat ggctgctgctg tagcgtataa tgcgttgatg caattttctat gcgcaccgt
 271 tctcggagca ctgtccgacc gctttggccg ccgcccagtc ctgctcgctt cgctacttgg agccactatc gactacgcga tcatggcgac
 361 cacaccctgc ctgtggatca ataattaatg aacatatata ctctatttaa tttttcttat ttattcgtaa tattgccata aaaaataaac
 451 attatttcta tattaactaa actgttaata ttgttaataa ataaacattt gtttatctaa aaaaataaat aatataaatc aagcaattac
 541 aatcttattt ttgaaaatac aataactcgt caattgctta atctagacat taagtttatt ttgtattaaa attgccaaaa cttgtgttaa
 631 taagtttcac cgaattgata ctttaagggt atcaatattg caacatggtt aatgaagctt atgttcttgg gcattgcata aattgtctat
 721 aataacttgt tatggatgat tgatggcaat gataaactta gtgacaatga taacgcgaag gagggtgaat atgtcattac aaactcaacc
 1 M S L Q T Q
 811 tgccaagaga gggttctatg ttaagccttt aagtaggctt tgcagctcgt taattagtgc tagtagtacg gtaagttatg ccaactgacg
 7 P A K R G F Y V K P L S M A C M L V I S A S S T V S Y A N S
 901 tccaatgatt gtgtattcac agtacaatag ttctaaatac tctttctacy attactattt agatttcctt aaacgtttta gaccaactcc
 37 A P M I V D S Q Y N S S K Y S F Y D Y Y L D F L K R F R P T
 991 aactccagtg ccaagccctg tgagaccggc tctgaactc gttcgtccga cccagccccc gattccggct ccaacgcctg tgccaacacc
 67 P T P V P S P V R P A P E L V R P T P A P I P A P T P V P T
 1081 ggcaccaatt agtgccggtt tatcaggtag ctatattgct ccagtagtgc catcagaggt gagacagcct gattacacaa gacgcgttca
 97 P A P I S G G I S G S Y I A P V S P S E V R Q P D Y T R R V
 1171 agccaatcta aaacgcaacc aacctgcacc aagtgcctgc acacgtacag gttatagtgt catggatagc tcaataaatt ctaatttgac
 127 Q A N L K R N Q P A P S A G T R T G Y S V M D T S N N S N L
 1261 atctaaatct tatggcaca ccaagagatg ttatgcggag cgtcttgaca acctaagaa caccattgat acacgtcaag ccaagtagg
 157 T S K F Y G T T E D G Y A E R L D N L K N T I D T R Q A K V
 1351 tgtgattgat acaggcatta accgcttcaa ccgagacttg gttggtgcaa atgtgcattg tacacagatt gagggtgttt ctgctggacg
 187 G V I D T G I N R F N R D L V G A N V H D T Q I E C V S A G
 1441 ttccacctgc tatacggcag aaaatgattc aggcattggt gaaatcccaa caacctctgc tagtggtagt catggcaacc aaatggcggc
 217 R S T C Y T P E N D S G I V E I P T T S A S G S H G N Q M A
 1531 tgtcatcgct ggttaacaac gcattgaccaa cgcgaataat tacggcagtg acagtattga tgcagcttca aatgggtgca accatttctt
 247 A V I A G N N G M T N A K I Y G S D S I D R R S N G G N H F
 1621 gatgatgcgt aagctgaacc aagaccatgg tgcgaagatt tttaaccaact cttgggggttc taacaacact gaccaatggt actacgatgc
 277 L M M R K L N Q D H G V K I F N N S W G S N N T D Q W Y I D
 1711 tcagcgccta aattacaatc ctactacagg acagattaat ccaaatcctt acagaaccag tattaccaat gctgaagtga ctttgcctgt
 307 A Q R L N Y N P T T G Q I N P N P Y R T S I T N A E V T L P
 1801 cattcatgat ctattatga atcgtgactc gcttatcatt aaagcaacag gtaacgaagg cttgaacgat gctcatgatg aaaccttagc
 337 V I H D L I M N R D S L I I K A T G N E G L N D A H D E N L
 1891 accgctcatg aacagcaact tcaaaaaagg ttctattact gtctcctcgc ctagagaaga ttttggtaaa gcgaatcatt gtggtcgaac
 367 A P L M N S N F K K G F I T P V S P R E D F G K A N H C G R
 1981 tgccgaatgg tgtgtatccg caacatcatc tacccaaaat tacgccaaag atggcagact gaggtagtat aagggtacat cacctgcaac
 397 T A E W C V S A T S S T Q N Y A N D G R L S S Y K G T S P A
 2071 cgctcgtgtg tccggcagcg cagtgcctgt gcaatctgct tatccttggg tgaataatga aaatatctct caaacattt tgggtactgc
 427 T A R V S G T A V L V Q S A Y P W H K N E N I S Q T I L G T
 2161 caaggatttc tcagagatta ctgccaatcc acctaatggc taccaaggac taagaaagggt tagtagattg ccattcgtgtt attacggctc
 457 A K D F S E I T A N S P N G Y Q G L R K V S R L P S G Y Y G
 2251 ttattacact gacaatcagg gtaatttcta tgttcctggc aatgtcaatt gggaaaaccg tcgaattgtc gctaataata acggcaagaa
 487 S Y Y T D N Q G N F Y V P G N V N W E N R R I V A N H N G K
 2341 cattacatgg gaagatggtt ggggtttggt agatccagaa gcggccgcta aagggttatg tggttcttat tgggataatg tgggaattga
 517 N I T W E D G W G L L D P E A A A K G Y G G F Y W D N V E L
 2431 cactaaaggc agccttttat ctgtatttcta caatgacctt aaagggtgata aaggctttac caaaaaagggt gaaggtaaac ttgtctttac
 547 D T K G T P L S V F Y N D L K G D K G F T K K G E G K L V F
 2521 tggttaataat agctataaag gcgactctgt catcgagggt ggttcactag aagtaaatgg taacaacggt ggttcaacca tgggtgttaa
 577 T G N N S Y K G D S V I E G G S L E V N G N N G G S T M V V
 2611 aggtgggtgaa ctaacaggtt atggtaatgt agctaatggt cgtcaaacag gtggttgggt taacaacgaa ggttaacctaa acatcagagg
 607 K G G E L T G Y G N V A N V R Q T G G W V N N E G N L M I R

Figure 1

2/7

2701 tgactacaac atcaacactc aacgtggcgt ggatgctggt ctaaaagctc aatttggcaa catgcttacc gtggacggta aggccaaact
 637 G D Y N I N T Q R G V D A G L K A Q F G N M L T V D G K A K
 2791 aggtgggtaca ctaaactcaa ctgggtgagac caaagatggt atcatcagca aatcaggtag cctgtagact gtacttcgtg ctaagcgtgg
 667 L G G T L N L T G E T K D G I I S K S G S R S T V L R A K R
 2881 tcttgaaggt caatttgaca attatcgttc aagcaaccga ttatttgaag taacaaatgt tgaatatag ccagaagtag acagaatagg
 697 G L E G Q F D N Y R S S N P L F E V T N V E Y T P E V D R N
 2971 cagagtggta ggtggttcac gcacgaacaa tgacgtgcaa gtaactgcca aacgtctaaq tgcaggcaat gttgtttatg gcatcagcat
 727 G R V V G G S R T N N D V Q V T A K R L S A G N V V Y G I S
 3061 gaatgacagt ggtagccgtg ttgcacaaaa cctagacaaa gtacttaatg atttagataa aaaacaagaa acacaaggtt cactgaccag
 757 M N D S G S R V A Q N L D K V L N D L D K K Q E T Q G S L T
 3151 tgatgagaag caatttgcta accgtgtatt cactggtttt gaaacatga attctggtgc agaactctaa ctttctacaa ctttctacaa
 787 S D E K Q F A N R V F T G F E N M N S G A E S K L S T V S T
 3241 ccgtgagcta tacaagcttg acccaacttt ctatgctgac agtgcatcaa acgcagtaga agacagtgtt aacctgcaa ccgaatttgg
 817 N R E L Y K L D P T F Y A D S A L N A V E D S A N H A T E F
 3331 taagcgtgtt agcgcccaa gaggtgtttg gggtaatatc agtcaccatg attatgatgt agaactagag catgctacaa gtgcacgtaa
 847 G K R V S A P R G V W G N I S H M D Y D V E L E H A T S A R
 3421 aggcaacaac attagtgttg gtgcaagcac tcaaaactga gccgacatta gtgttgggtg acaacttgat gtaagttaac ttgacttggg
 877 K G N N I S V G A S T Q T A A D I S V G A Q L D V S K L D L
 3511 agaactgttt tatgttattg gcaacaaaac caaaactgac agcattggtt tgactgttgg tgcttctaag aagtgtgggt atgcctatct
 907 E E S V Y G I G N K T K T D S I G L T V G A S K K L G D A Y
 3601 atcaggttgg gtaaaagtg ccaaagttga tacagaagcg aaccgtggtg aaaactctaa caaagttaga tacaatggta agctatatgg
 937 L S G W V K G A K V D T E A N R G E N S N K V E Y N G K L Y
 3691 tgctggtatc caagcgggta caaacattga tactgcatcg ggcgtgagtg tacaacctta tgctttgttt aacctcagc agtacaaaa
 967 G A G I Q A G T N I D T A S G V S V Q P Y A F V N H Q Q Y K
 3781 cgtggttagc ttcaatgagc gtcttaacgt tgttgacgac atcgaagcaa aacaaactca ggtaggtgtg ggtgctgata tgggtgtcca
 997 N D G S F N D G L N V V D D I E A K Q T Q V G V G A D M V F
 3871 agcaacacct gctctacagc ttactggttg tgtgcaagtt gctcacgttg ttacgctgga caccaacctta gacactcgtt atgttggtag
 1027 Q A T P A L Q L T G W V Q V A H A V S R D T N L D T R Y V G
 3961 agcgacagat gtacagtatg gcacttggga tactgacaaa accaaatggt cagccaaaggt tgggtgtaac tataatgtga caccaaacag
 1057 T A T D V Q Y G T W D T D K T K W S A K V G A N Y N V T P N
 4051 ccaagtgggt ctaaaactaca gctacacagg tagtggcgat tcagatgctt cccaagtggg tgtgagcttc accagcaagt tctaattcat
 1087 S Q V G L N Y S Y T G S G D S D A S Q V G V S F T S K F -
 4141 taataaggca acaaaaaca gcacaatttc ggttgtgctg ttttttgtga tgccgagcgt aaaattttcc caaaaaaagc gtgataatta
 4231 ccacgctttt ttattgcata ttgcaaaata gtattgcatt tatgggttgt taagcaaccc gtccaaatcc cccctaaaca actccacccc
 4321 aatcgggtgct aacttgtttt gccacaggct cgtcaatgtg tcggcatcat caaccattac cgac

Figure 1 (continued)

3/7

1 tgggcagata acccatcaaa gacccaaagc aacccataaa tcaaaaaaac acrrgtatatt tgtgtaatat cttgtacac tttacaagtg
91 tttttacttt gaaagcaact cagaaagttaa taatgaaaaa atccgccttt gccaaatact cagcacttgc cctaagtgtt gggatgtgcc
1 M K K S A F A K Y S A L A L H V G M C
181 tgcacaccgc ttacgccaag gagtttagcc aagtcatcat ttttggggac agcttgtccg atacaggtcg cctaaaagat atgtgcqccc
20 L H T A Y A X E F S Q V I I F G D S L S D T G R L K D H V A
271 gaaaagatgg cacccttggc aacaccttac agccatcttt taccaccaac cccgaccctg tatggtcaag cttatitggc caaagtatat
50 R K D G T L G N T L Q P S F T T N P D P V W S S L F A Q N A I K G A V T
361 gcaaaaccgc cagtgcacac acgacctaca atcccaactgg cactaaactat gccgtgggcg gagctcgctc tggctcggag gtcaattgga
80 G K T A S A N T P Y N R T G T N Y A V G G A R S G S E V N W
451 atgtttttgt gaatgtacc tccaccaaaa cgcacaatcac cgaccatttg accgcacag gtggcaaac cgaccctaatt accctgtatg
110 N G F V N V P S T K T Q I T D H L T A T G G K A D F N T L Y
541 ccatttggat tggctctaatt gacttaattt cagcttctca agccaccaca acagccgaag ccaaaaacgc cattaaaggt gcggttaactc
140 A I W I G S N D L I S A S Q A T T T A E A Q N A I K G A V T
631 gcaccgtgat agacatcgaa acactcaatc aagcaggggc gacaaccatt ttgtgtccaa atgtgcctga tttgagcctc acgccccgag
170 R T V I D I E T L N Q A G A T T I L V P N V P D L S L T P R
721 ccattctatgg cgaagccctc atggcaggcg tgcaagacaa agccaaaact gcctcaagtc tgtataatag cggctctgtt gaagcattaa
200 A I Y G E S L M A G V Q D K A K L A S S L Y N S G L F E A L
811 atcaatccac cgcacaatc atccctggca acacctttgc cctactccaa gaagcgacca caaataaaga agccttttgt tttaaaaaca
230 N Q S T A N I I P A N T F A L L Q E A T T N K E A F G F K N
901 cgcgaaggcg ggcgtgtcaa atgcccgcctc gtaccacagg ggcggatgat gtggttctca cttccttggc atgtaccaaa gccaatctta
260 T Q G V A C Q M P A R T T G A D D V A S T S L A C T K A N L
991 tagaaaaacg gcaaatgac acctacgcct ttgccgatga cattcaccca tccggacgca cgcaccgcat tttggcacag cattaccgct
290 I E N G A N D T Y A F A D D I H P S G R T H R I L A Q Y Y R
1081 ctatctgga cgcctctact cacatgggta aactctcagg cgagcttgc aaaaacaggt cagccacga cgcctatgtt taccgtcagc
320 S I M D A P T H M G K L S G E L V K T G S A H D R H V Y K N
1171 ttgacaggct tagtggctca cagcacagca tttgggcaaa cgtccatgcc agcgaccgta cgcacccac cacccaaatc ggcctggagc
350 L D R L S G S Q H S I W A N V H A S D R T D P T T Q I G L D
1261 tggcagggtc atcaagccat acagggggcg atctgagcca ccaaaaccaa gattatgtgc tggatgacac cctatcatca gatgtcaaaa
380 V A G S S S H T G A Y L S H Q N Q D Y V L D D T L S S D V K
1351 ccattggcat ggggtgttat cctgcctatg acatcgccaa tgtccgtcta aaaggcggtg caggtatcga cgcatttagc gtgatacgc
410 T I G M G L Y H R H D I G N V R L K G V A G I D R L S V D T
1441 accgcatat cgactgggag ggggcaagcc gttcgacac gccagacac accgcagac gtttctatgc agggctacaa gccagctatg
440 H R H I D W E G A S R S H T A D T T A R R F H A G L Q A S Y
1531 gcatagacat gggcaaaagc accgtgcctc cgttatcgg cgtacatgcc caaaaagta aagtgcgtga tttgtagag aatgagccta
470 G I D M G K A T V R P L I G V H A Q K V K V R D L V E N E P
1621 ccctatccac cgcctatgct tttggcgagc aagaacaaaa gtccctacaa ggcgagattg gcgtcgtatg ggcctatcgg attagccctg
500 T L S T A M R F G E Q E Q K S L Q G E I G V D V A Y P I S P
1711 ctttgactct gacggggcggt atcgctcacg ctcctagatt taacgatgat gaacyacca ttaatgccac tttacctcc attcgtgaat
530 A L T L T G G I A H A H E F N D D E R T I N A T L T S I R E
1801 acacgaaggg ctttaataca agcgttagca ccgacaaatc tcacgccacc accgctcctc tggcgtaga agggcaactt ggcgaaggca
560 Y T K G F N T S V S T D K S H A T T A H L G V Q G Q L G K A
1891 atattcatgc agcggttcac gccacccacc aagacagcga tacagacgtg ggtggttcgc ttggggttcg cttgatgttt tgaattgctt
590 N I H A G V H A T H Q D S D T D V G G S L G V R L M F -
1981 ttaaagataa aaagtgttat catgccactt tttattttgc caaaaatcta tgtttgagta catcaagcc tttcacatca tgcctatgcg
2071 atgataagct gtcaaacatg ag

Figure 2

4/7

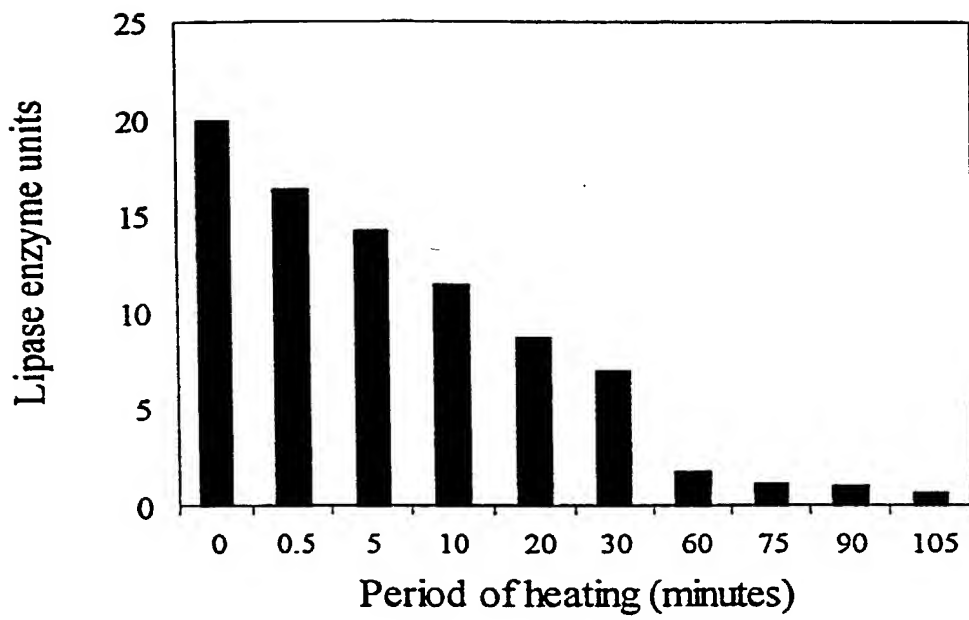
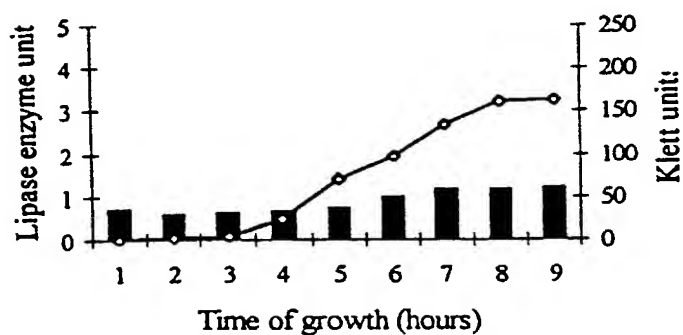


Figure 3

5/7

(i) Dalton 2d / *M. bovis*



(ii) pMB1 / MC1061

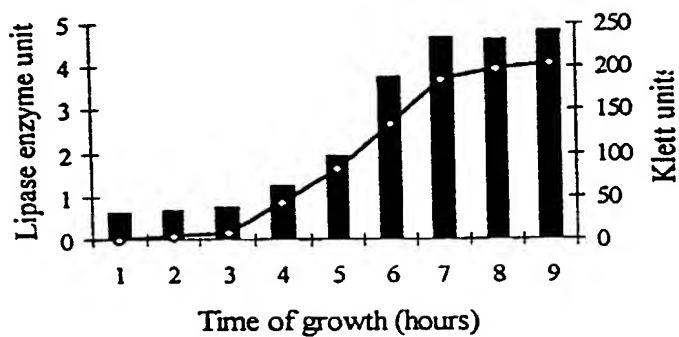


Figure 4

1 atgagaacgt tattttcaga tgaattgttt agagcgattc gtgtagatgg aaattcatcg catggtaaga tatctgaatt ttatggaag
M R T L F S D E L F R A I R V D G N S S H G K I S E F Y G K

91 tctgttgatt caaaattagc ctcaagaata ttgcacaat atcacgaaga ttgacgagc aaattgtcaa ctcaagaata tttattata
S V D S K L A S R I F A Q Y H E D L T S K L S T Q N N F I I

181 tctaaagata attaatcaa ccttttctaa cacacgagg ~~aaacacat~~ tatgtccaat atcaatgtaa ttaaatctaa tttcaagca
1 S K D N - M S N I N V I K S N I Q A

271 ggcttgatt caacaaagtc tggattaaaa aatctttact tggctattcc caaagattat gatccgcaa aaggtgggac tttaatgat
14 G L N S T K S G L K N L Y L A I P K D Y D P Q K G G T L N D

361 tttattaaag ctgctgatga attaggtatt gctcgtttag cagaagagcc taatcacact gaaacagcaa aaaaatctgt tgacacagta
44 F I K A A D E L G I A R L A E E P N H T E T A K K S V D T V

451 aatcagtttc tctctctcac acaactcgtt attgctattt ctgcaacaaa attagaaaag ttcttcaaaa aacattctac caataagtta
74 N Q F L S L T Q T G I A I S A T K L E K F L Q K H S T N K L

541 gccaaaggtg tagacagtg agaaaaatatt gatcgttaat taggttaagc aagtaatgta ttatcaacat taagctcttt ttgggcact
104 A K G L D S V E N I D R K L G K A S N V L S T L S S F L G T

631 gcattagcgg gtatagaact tgattcttta atcaaaaaag gtgatgtgac accgtatgct ttggctaaag ctagtattga ctgtattat
134 A L A G I E L D S L I K K G D A A P D A L A K A S I D L I N

721 gagataattg gtaattctatc tcagagtact caaacgattg aagcattttc ttcacagtta gcaaatgttag gtcttactat atcgcaggct
164 E I I G N L S Q S T Q T I E A F S S Q L A K L G S T I S Q A

811 aaagcttctt ctaatatagg aaacaagttg caaaacttaa atttttctaa acaaatctt gggttggaaa taattactgg ttgctatca
194 K G F S N I G N K L Q N L N F S K T N L G L E I I T L G L L S

901 ggcattttct caggctttgc tttagcggat aaaaatgcat cgactggcaa aaaagtgtgt gcaggttttg aattaagcaa tcaagttatt
224 G I S A G F A L A D K N A S T G K K V A A G F E L S N Q V I

991 ggtaagttaa caaaagcaat ttcttcatat gtttttagcac aacgtgttgc tgctgtgtca tcaactactg gtgctgttgc tgccttaatt
254 G N V T K A I S S Y V L A Q R V A A G L S T T G A V A A L I

1081 acttcatcga ttatgtttgc aattagtcct ttggcattta tgaatgcagc agataaattc aatcatgcta atgctcttga tgagtttga
284 T S S I M L A I S P L A F M N A A D K F N H A N A L D E F A

1171 aaacaattcc gaaaatttgg ctatgatggg gatcatttat tggctgaata tcagcgtggt gtgggtacta ttgaagcttc attaatca
314 K Q F R K F Q D G D H L L A E Y Q R G V G T I E A S L T T

1261 attagtaagg cattaggtgc agtttctgct ggtgtttccg ctgctgtgct aggatcgtct gttggtacac cgattgcact attagttga
344 I S T A L G A V S A G V S A A A V G S A V G T P I A L L V A

1351 ggtgttacag gattgatctc tggaaattta gaagcgtcta aacaggcaat gtttgaagt gttgctaacc gtttacaagg taaatttta
374 G V T G L I S G I L E A S K Q A M F E S V A N R L Q G K I L

1441 gagtgggaaa agcaaaattg cggctcagaac tattttgata aaggtcatga ttctcgttat gctgcttatt tagctaataa cttaaaattt
404 E W E K Q N G G Q N Y F D K G Y D S R Y A A Y L A N N L K F

1531 ttgtctgagc taaataaaga gttggaagct gaacgtgtta ttgcaatcac ccaacaacgt tgggataata atattgttga gttagcaggt
434 L S E L N K E L E A E R V I A I T Q Q R W D N N I G E L A G

1621 attaccaaat tgggtgaagc cattaaagac ggaagaagctt atgcagatgc ttttgaagat ggcaagaag ttgaagctgg ttcaatatt
464 I T K L G E R I K S G K A Y A D A F E D G K K V E A G S N I

1711 actttggatg ctaaaactgg tatcatagac attagtaatt caaatgggaa aaaaacgcaa gcgttgcatt tcacttcgcc tttgttaaca
494 T L D A K T G I I D I S N S N G K K T Q A L H F T S P L L T

1801 gcaggaaact aatcacgtga acgtttaact aatggttaat actcttatat taataagtta aaattcggac gtgtaaaaaa ctggcaagt
524 A G T E S R E R L T N G K Y S Y I N K L K F G R V K N W Q V

1891 acagatggag aggttagttc taaattagat ttctctaaag ttattcagcg tgtagccgag acagaaggca cagacgagat tggcttaata
554 T D G E A S S K L D F S K V I Q R V A E T E G T D E I G L I

1981 gtaaatgcaa aagctggcaa tgacgatatc tttgttggtc aaggttaaat gaattattgat ggtggagatg gacacgacgt tgcctctat
584 V N A K A G N D D I F V G Q G K M N I D G G D G H D R V F Y

2071 agtaaaagac gaggatttgg taattattact gtatagtgta cgagtgcac agaagcaggg agttatacag ttaactgtaa ggttgcctga
614 S K D G G F G N I T V D G T S A T E A G S Y T V N R K V A R

2161 ggtgatattc accatgaagt tgtgaagcgt caagaacca aggtgggtaa acgtactgaa actatccagt atcgtgatta tgaattaa
644 G D I Y H E V V K R Q E T K V G K R T E T I Q Y R D Y E L R

2251 aaagtgggt atgttatca gtctaccgat aatttgaaat cagtgaaga agtaattggt tctcaattta atgatgatt caaagcttct
674 K V G Y G Y Q S T D N L K S V E E V I G S Q F N D V F K G S

2341 aaattcaagc acatattcca tagtgggtgaa ggtgatgatt taactcgatg tgggtgctgt gacgacgct tgttgggtgg taaaggcaac
704 K F N D I F H S G E G D D L L D G G A G D D R L F G G K G N

Figure 5

WO 01/16172

PCT/AU00/01048

7/7

```

2431 gatcgacttt ctggagatga aggcgatgat ttactcgatg gcggtctctg tgatgatgta ttaaatgggtg gtgctggtaa tgatgtctat
734 D R L S G D E G D D L L D G G S G D D V L N G G A G N D V Y

2521 atcttttcgga aagggtgatgg taatgatact ttgtacgatg gcacggggcaa tgataaatta gcatttgcag atgcaaatat atctgatatt
764 I F R K G D G N D T L Y D G T G N D K L A F A D A N I S D I

2611 atgattgaac gtaccaaaaga ggggtattata gttaaacgaa atgatcattc aggtagtatt aacataccas gatggtagac aacatcaaat
794 M I E R T K E G I I V K R N D H S G S I N I P R W Y I T S H

2701 ttacaaaatt atcaaaagtaa taaaacagat cataaaattg agcaactaat tggtaaagat ggtagtata tcacttccga tcaaattgat
824 L Q N Y Q S N K T D H K I E Q L I G K D G S Y I T S D Q I D

2791 aaaattttgc aagataagaa agatgggtaca gtaattacat ctcaagaatt gaaaaagctt gctgatgaga ataagagcca aaaattatct
854 K I L Q D K K D G T V I T S Q E L K K L A D E N K S Q K L S

2881 gcttcggaca ttgcaagtag cttaataaag ctagtgggt caatggcact atttgggtaca gcaaatagtg tgagtcttaa cgccttcacg
884 A S D I A S S L N K L V G S M A L F G T A N S V S S N A L Q

2971 ccaattacac aaccaactca aggaattttg gctccaagtg tttagtgtatt taatttacta gacaatatca ccaccatcat cattgggtat
914 P I T Q P T Q G I L A P S V -

3061 agattatgaa actagtata tgggtgggtga tacttcttta attagactta atttacaac ccttaatagt aatttagtta tgatagatta
H G G D T S L I R L N L Q T L N S N L V M I D

3151 tgctcaacas cctgtcttat ctgctctggt tatccttgc aaatactatg gtatttctgc aagtcacga gacattatgc atcgactggc
Y A Q Q P A L S A L V I L A K Y Y G I S A S P A D I M H R L

3241 aaaaaagtgtg
A K K L

```

Figure 5 (continued)

Attorney Docket No. 20-02

JOINT INVENTORS' DECLARATION FOR PATENT APPLICATION
AND POWER OF ATTORNEY

As the below named inventors, we hereby declare that:

Our residences, post office addresses and citizenship are as stated below our names.

We believe that we are the original, first and joint inventors of the subject matter which is claimed and for which a patent is sought on the invention entitled: Vaccine Antigens of Moraxella.

the specification of which:

_____ is attached hereto;

X was filed on February 28, 2002 as Application Serial No. 10/069,799.

We hereby authorize our legal representative to add reference to the Serial No. and/or filing date of the above-referenced application to this declaration.

We hereby state that we have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

Prior Foreign Application(s)

We hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application(s) for patent or inventor's certificate having a filing date before that of the application to which priority is claimed:

| Country | Application No. | Date of Filing (day,month,year) | Date of Issue (day,month,year) | Priority Claimed 35 U.S.C.119 |
|-----------|-----------------|------------------------------------|-----------------------------------|----------------------------------|
| Australia | PQ2571 | 31 August 1999 | | Yes <u>X</u> No <u> </u> |

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-00 (1) Full Name of

First Inventor:

Jacinta FARN

Residence:

~~Essendon, Victoria~~ Yarck, Victoria AUK

Citizenship:

Australian

Post Office Address:

~~61 Glass Street~~ 7507 Maroondah Highway
~~Essendon, Victoria~~ 3040 Yarck, Victoria . 3719
AUSTRALIA

(1) Signature

J. Farn

Date 3/7/02

2-00 (2) Full Name of

Second Inventor:

Richard STRUGNELL

Residence:

Hawthorn, Victoria AUK

Citizenship:

Australian

Post Office Address: 11 Salisbury Grove

Hawthorn, Victoria 3122
AUSTRALIA

(2) Signature

R. Strugnell

Date 3.7.02

3-00 (3) Full Name of

Third Inventor:

Jan TENNENT

Residence:

Hawthorn East, Victoria AUK

Citizenship:

Australian

Post Office Address:

6 Campbell Grove
Hawthorn East, Victoria 3123
AUSTRALIA

(3) Signature

J. Tennent

Date 9 July 2002

```

<400> 1
Met Ser Leu Gln Thr Gln Pro Ala Lys Arg Gly Phe Tyr Val Lys Pro
  1                    5                10                15

Leu Ser Met Ala Cys Met Leu Val Ile Ser Ala Ser Ser Thr Val Ser
      20                25                30

Tyr Ala Asn Ser Ala Pro Met Ile Val Asp Ser Gln Tyr Asn Ser Ser
      35                40                45

Lys Tyr Ser Phe Tyr Asp Tyr Tyr Leu Asp Phe Leu Lys Arg Phe Arg
      50                55                60

Pro Thr Pro Thr Pro Val Pro Ser Pro Val Arg Pro Ala Pro Glu Leu
      65                70                75                80

Val Arg Pro Thr Pro Ala Pro Ile Pro Ala Pro Thr Pro Val Pro Thr
      85                90                95

Pro Ala Pro Ile Ser Gly Gly Ile Ser Gly Ser Tyr Ile Ala Pro Val
      100                105                110

Ser Pro Ser Glu Val Arg Gln Pro Asp Tyr Thr Arg Arg Val Gln Ala
      115                120                125

Asn Leu Lys Arg Asn Gln Pro Ala Pro Ser Ala Gly Thr Arg Thr Gly
      130                135                140

Tyr Ser Val Met Asp Thr Ser Asn Asn Ser Asn Leu Thr Ser Lys Phe
      145                150                155                160

Tyr Gly Thr Thr Glu Asp Gly Tyr Ala Glu Arg Leu Asp Asn Leu Lys
      165                170                175

Asn Thr Ile Asp Thr Arg Gln Ala Lys Val Gly Val Ile Asp Thr Gly
      180                185                190

Ile Asn Arg Phe Asn Arg Asp Leu Val Gly Ala Asn Val His Asp Thr
      195                200                205

```

2/14

Gln Ile Glu Cys Val Ser Ala Gly Arg Ser Thr Cys Tyr Thr Pro Glu
 210 215 220
 Asn Asp Ser Gly Ile Val Glu Ile Pro Thr Thr Ser Ala Ser Gly Ser
 225 230 235 240
 His Gly Asn Gln Met Ala Ala Val Ile Ala Gly Asn Asn Gly Met Thr
 245 250 255
 Asn Ala Lys Ile Tyr Gly Ser Asp Ser Ile Asp Arg Arg Ser Asn Gly
 260 265 270
 Gly Asn His Phe Leu Met Met Arg Lys Leu Asn Gln Asp His Gly Val
 275 280 285
 Lys Ile Phe Asn Asn Ser Trp Gly Ser Asn Asn Thr Asp Gln Trp Tyr
 290 295 300
 Tyr Asp Ala Gln Arg Leu Asn Tyr Asn Pro Thr Thr Gly Gln Ile Asn
 305 310 315 320
 Pro Asn Pro Tyr Arg Thr Ser Ile Thr Asn Ala Glu Val Thr Leu Pro
 325 330 335
 Val Ile His Asp Leu Ile Met Asn Arg Asp Ser Leu Ile Ile Lys Ala
 340 345 350
 Thr Gly Asn Glu Gly Leu Asn Asp Ala His Asp Glu Asn Leu Ala Pro
 355 360 365
 Leu Met Asn Ser Asn Phe Lys Lys Gly Phe Ile Thr Val Ser Ser Pro
 370 375 380
 Arg Glu Asp Phe Gly Lys Ala Asn His Cys Gly Arg Thr Ala Glu Trp
 385 390 395 400
 Cys Val Ser Ala Thr Ser Ser Thr Gln Asn Tyr Ala Asn Asp Gly Arg
 405 410 415
 Leu Ser Ser Tyr Lys Gly Thr Ser Pro Ala Thr Ala Arg Val Ser Gly
 420 425 430
 Thr Ala Val Leu Val Gln Ser Ala Tyr Pro Trp Met Lys Asn Glu Asn
 435 440 445
 Ile Ser Gln Thr Ile Leu Gly Thr Ala Lys Asp Phe Ser Glu Ile Thr
 450 455 460
 Ala Asn Ser Pro Asn Gly Tyr Gln Gly Leu Arg Lys Val Ser Arg Leu
 465 470 475 480
 Pro Ser Gly Tyr Tyr Gly Ser Tyr Tyr Thr Asp Asn Gln Gly Asn Phe
 485 490 495
 Tyr Val Pro Gly Asn Val Asn Trp Glu Asn Arg Arg Ile Val Ala Asn
 500 505 510

WO 01/16172

PCT/AU00/01048

3/14

| | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | Asn | Gly | Lys | Asn | Ile | Thr | Trp | Glu | Asp | Gly | Trp | Gly | Leu | Leu | Asp | 515 | 520 | 525 |
| Pro | Glu | Ala | Ala | Ala | Lys | Gly | Tyr | Gly | Gly | Phe | Tyr | Trp | Asp | Asn | Val | 530 | 535 | 540 |
| Glu | Leu | Asp | Thr | Lys | Gly | Thr | Pro | Leu | Ser | Val | Phe | Tyr | Asn | Asp | Leu | 545 | 550 | 555 |
| Lys | Gly | Asp | Lys | Gly | Phe | Thr | Lys | Lys | Gly | Glu | Gly | Lys | Leu | Val | Phe | 565 | 570 | 575 |
| Thr | Gly | Asn | Asn | Ser | Tyr | Lys | Gly | Asp | Ser | Val | Ile | Glu | Gly | Gly | Ser | 580 | 585 | 590 |
| Leu | Glu | Val | Asn | Gly | Asn | Asn | Gly | Gly | Ser | Thr | Met | Val | Val | Lys | Gly | 595 | 600 | 605 |
| Gly | Glu | Leu | Thr | Gly | Tyr | Gly | Asn | Val | Ala | Asn | Val | Arg | Gln | Thr | Gly | 610 | 615 | 620 |
| Gly | Trp | Val | Asn | Asn | Glu | Gly | Asn | Leu | Asn | Ile | Arg | Gly | Asp | Tyr | Asn | 625 | 630 | 635 |
| Ile | Asn | Thr | Gln | Arg | Gly | Val | Asp | Ala | Gly | Leu | Lys | Ala | Gln | Phe | Gly | 645 | 650 | 655 |
| Asn | Met | Leu | Thr | Val | Asp | Gly | Lys | Ala | Lys | Leu | Gly | Gly | Thr | Leu | Asn | 660 | 665 | 670 |
| Leu | Thr | Gly | Glu | Thr | Lys | Asp | Gly | Ile | Ile | Ser | Lys | Ser | Gly | Ser | Arg | 675 | 680 | 685 |
| Ser | Thr | Val | Leu | Arg | Ala | Lys | Arg | Gly | Leu | Glu | Gly | Gln | Phe | Asp | Asn | 690 | 695 | 700 |
| Tyr | Arg | Ser | Ser | Asn | Pro | Leu | Phe | Glu | Val | Thr | Asn | Val | Glu | Tyr | Thr | 705 | 710 | 715 |
| Pro | Glu | Val | Asp | Arg | Asn | Gly | Arg | Val | Val | Gly | Gly | Ser | Arg | Thr | Asn | 725 | 730 | 735 |
| Asn | Asp | Val | Gln | Val | Thr | Ala | Lys | Arg | Leu | Ser | Ala | Gly | Asn | Val | Val | 740 | 745 | 750 |
| Tyr | Gly | Ile | Ser | Met | Asn | Asp | Ser | Gly | Ser | Arg | Val | Ala | Gln | Asn | Leu | 755 | 760 | 765 |
| Asp | Lys | Val | Leu | Asn | Asp | Leu | Asp | Lys | Lys | Gln | Glu | Thr | Gln | Gly | Ser | 770 | 775 | 780 |
| Leu | Thr | Ser | Asp | Glu | Lys | Gln | Phe | Ala | Asn | Arg | Val | Phe | Thr | Gly | Phe | 785 | 790 | 795 |
| Glu | Asn | Met | Asn | Ser | Gly | Ala | Glu | Ser | Lys | Leu | Ser | Thr | Val | Ser | Thr | 805 | 810 | 815 |

| | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|------|-----|--|--|------|
| Asn | Arg | Glu | Leu | Tyr | Lys | Leu | Asp | Pro | Thr | Phe | Tyr | Ala | Asp | Ser | Ala | | | |
| | | | | | | | 820 | | | | | | | 825 | | | | 830 |
| Leu | Asn | Ala | Val | Glu | Asp | Ser | Ala | Asn | His | Ala | Thr | Glu | Phe | Gly | Lys | | | |
| | | | | | | | 835 | | | | | | | 840 | | | | 845 |
| Arg | Val | Ser | Ala | Pro | Arg | Gly | Val | Trp | Gly | Asn | Ile | Ser | His | His | Asp | | | |
| | | | | | | | 850 | | | | | | | 855 | | | | 860 |
| Tyr | Asp | Val | Glu | Leu | Glu | His | Ala | Thr | Ser | Ala | Arg | Lys | Gly | Asn | Asn | | | |
| | | | | | | | 865 | | | | | | | 870 | | | | 875 |
| Ile | Ser | Val | Gly | Ala | Ser | Thr | Gln | Thr | Ala | Ala | Asp | Ile | Ser | Val | Gly | | | |
| | | | | | | | 885 | | | | | | | 890 | | | | 895 |
| Ala | Gln | Leu | Asp | Val | Ser | Lys | Leu | Asp | Leu | Glu | Glu | Ser | Val | Tyr | Gly | | | |
| | | | | | | | 900 | | | | | | | 905 | | | | 910 |
| Ile | Gly | Asn | Lys | Thr | Lys | Thr | Asp | Ser | Ile | Gly | Leu | Thr | Val | Gly | Ala | | | |
| | | | | | | | 915 | | | | | | | 920 | | | | 925 |
| Ser | Lys | Lys | Leu | Gly | Asp | Ala | Tyr | Leu | Ser | Gly | Trp | Val | Lys | Gly | Ala | | | |
| | | | | | | | 930 | | | | | | | 935 | | | | 940 |
| Lys | Val | Asp | Thr | Glu | Ala | Asn | Arg | Gly | Glu | Asn | Ser | Asn | Lys | Val | Glu | | | |
| | | | | | | | 945 | | | | | | | 950 | | | | 955 |
| Tyr | Asn | Gly | Lys | Leu | Tyr | Gly | Ala | Gly | Ile | Gln | Ala | Gly | Thr | Asn | Ile | | | |
| | | | | | | | 965 | | | | | | | 970 | | | | 975 |
| Asp | Thr | Ala | Ser | Gly | Val | Ser | Val | Gln | Pro | Tyr | Ala | Phe | Val | Asn | His | | | |
| | | | | | | | 980 | | | | | | | 985 | | | | 990 |
| Gln | Gln | Tyr | Lys | Asn | Asp | Gly | Ser | Phe | Asn | Asp | Gly | Leu | Asn | Val | Val | | | |
| | | | | | | | 995 | | | | | | | 1000 | | | | 1005 |
| Asp | Asp | Ile | Glu | Ala | Lys | Gln | Thr | Gln | Val | Gly | Val | Gly | Ala | Asp | Met | | | |
| | | | | | | | 1010 | | | | | | | 1015 | | | | 1020 |
| Val | Phe | Gln | Ala | Thr | Pro | Ala | Leu | Gln | Leu | Thr | Gly | Gly | Val | Gln | Val | | | |
| | | | | | | | 1025 | | | | | | | 1030 | | | | 1035 |
| Ala | His | Ala | Val | Ser | Arg | Asp | Thr | Asn | Leu | Asp | Thr | Arg | Tyr | Val | Gly | | | |
| | | | | | | | 1045 | | | | | | | 1050 | | | | 1055 |
| Thr | Ala | Thr | Asp | Val | Gln | Tyr | Gly | Thr | Trp | Asp | Thr | Asp | Lys | Thr | Lys | | | |
| | | | | | | | 1060 | | | | | | | 1065 | | | | 1070 |
| Trp | Ser | Ala | Lys | Val | Gly | Ala | Asn | Tyr | Asn | Val | Thr | Pro | Asn | Ser | Gln | | | |
| | | | | | | | 1075 | | | | | | | 1080 | | | | 1085 |
| Val | Gly | Leu | Asn | Tyr | Ser | Tyr | Thr | Gly | Ser | Gly | Asp | Ser | Asp | Ala | Ser | | | |
| | | | | | | | 1090 | | | | | | | 1095 | | | | 1100 |
| Gln | Val | Gly | Val | Ser | Phe | Thr | Ser | Lys | Phe | | | | | | | | | |
| | | | | | | | 1105 | | | | | | | 1110 | | | | |

WO 01/16172

PCT/AU00/01048

5/14

<210> 2
<211> 4384
<212> DNA
<213> Moraxella bovis

<400> 2
 ttctcatgtt tgacagctta tcatcgataa gctttaatgc ggtagtttat cacagttaaa 60
 ttgctaacgc agtcaggcac cgtgtatgaa atctaacaat gcgctcatcg tcatcctcgg 120
 caccgtcacc ctggatgctg taggcatagg cttggttatg ccggtactgc cgggcctctt 180
 gcgggatatc gtccattccg acagcatcgc cagtcaactat ggcgtgctgc tagcgctata 240
 tgcgttgatg caatttctat gcgcaccctg tctcggagca ctgtccgacc gctttggccg 300
 ccgcccagtc ctgctcgctt cgctacttgg agccactatc gactacgcga tcatggcgac 360
 cacaccctgc ctgtggatca ataattaatg aacatatata ctctatttaa tatttcttat 420
 ttattcgtaa tattgccata aaaataatc attatttcta tattaactaa actgttaata 480
 tttgtaaaata ataaacattt gtttatctaa aaaaaaat aatataaatc aagcaattac 540
 aatcttattt ttgaaaatac aataatactg caattgctta atctagacat taagtttatt 600
 tttgattaaa attgccaaaa cttgtgtaaa taagtttcac cgaattgata ctttaaggg 660
 atcaatattg caacatggta aatgattgct atgttggtgg gcattgcata aattgtctat 720
 aataacttgt tatggatgat tgatggcaat gataaactta gtgacaatga taaacgcaaa 780
 gaggtgtaat atgtcattac aaactcaacc tgccaagaga gggttctatg ttaagccttt 840
 aagtatggct tgcattgctg taatttagtg tagtagtacg gtaagttagt ccaactcagc 900
 tccaatgatt ttgtattcac agtacaatag ttctaaatac tctttctacg attactattt 960
 agatttctct aaacgtttta gaccaactcc aactccagtg ccaagccctg tgagaccggc 1020
 tctgaactc gtctgtccga cccagcccc gattccggct ccaacgcctg tgccaacacc 1080
 ggcaccaatt agtggcggta tatcaggtag ctatattgct ccagtatcgc catcagaggt 1140
 gagacagcct gattacacaa gacgcgttca agccaatcta aaacgcaacc aacctgcacc 1200
 aagtgtctgg acacgtacag gttatagtg tagtgatac tcaaataatt ctaatttgac 1260
 atctaaattt tatggcacia ccgaagatgg ttatcccgag cgtcttgaca acctaaagaa 1320
 caccattgat acacgtcaag ccaaagtagg tgtgattgat acaggcatta accgcttcaa 1380
 ccgagacttg gttggtgcaa atgtgcatga tacacagatt gagtgtgttt ctgctggacg 1440
 ttccacctgc tatacgccag aaaatgattc aggcattggt gaaatcccaa caacctctgc 1500
 tagtggtagt catggcaacc aaatggcggc tgtcatcgtt ggtaacaacg gcatgacca 1560
 cgccaaaatt tacggcagtg acagtattga tcgacgttca aatgggtgga accatttctt 1620
 gatgatgcgt aagctgaacc aagaccatgg tgtcaagatt tttaacaact cttgggggtt 1680
 taacaacact gaccaatggt actacgatgc tcagcgccca aattacaatc ctactacagg 1740
 acagattaat ccaaactcct acagaaccag tattaccaat gctgaagtga ctttgctgt 1800
 cattcatgat cttattatga atcgtgactc gcttatcatt aaagcaacag gtaacgaagg 1860
 cttgaacgat gtcctgatg aaaacctagc accgctcatg aacagcaact tcaaaaaagg 1920
 tttcattact gtctcctcgc cttagagaaga ttttggtaaa gcgaatcatt gtggctgaac 1980
 tgccgaatgg tgtgtatccg caacatcac tacccaaat tacgccaacg atggcagact 2040
 gagtagctat aagggtacat cacctgcaac cgctcgtgtg tccggcacgg cagtgtcgt 2100
 gcaatctgct tatccttgga tgaaaaatga aaatatctct caaaccattt tgggtactgc 2160
 caaggatttc tcagagatta ctgccaattc acctaatggc taccaaggac taagaaagg 2220
 tagtagattg ccatctgggt attacggctc ttattacact gacaatcagg gtaatttcta 2280
 tgttcctggc aatgtcaatt gggaaaaacc tcgaattgtc gctaatacata acggcaagaa 2340
 cattactatg gaagatgggt ggggtttgtt agatccagaa gcggccgcta aagggtatgg 2400
 tggtttctat tgggataatg tggaattaga cactaaaggc acgcctttat ctgtattcta 2460
 caatgacctt aaaggatgata aaggctttac caaaaaaggg gaaggtaaac ttgtctttac 2520
 tggtaataat agctataaag gcgactctgt catcgagggt ggttcactag aagtaaatgg 2580
 taacaacggg ggttcaacca tgggtgttaa aggtgggtgaa ctaacagggt atggtaatgt 2640
 agctaattgt cgtcaaacag gtggttgggt taacaacgaa ggtaacctaa acatcagagg 2700
 tgactacaac atcaacactc aacgtggcgt ggatgctggt ctaaaagctc aatttggcaa 2760
 catgcttacc gtggacggta aggccaactc aggtgggtaca ctaaatctaa ctgggtgagc 2820
 caaagatggg atcatcagca aatcaggtag ccgtgagact gtacttctgt ctaagcgtgg 2880
 tcttgaagggt caatttgaca attatcgttc aagcaaccca ttatttgaag taacaaatgt 2940

6/14

```

tgaatatacg ccagaagtag acagaaatgg cagagtggta ggtgggttcac gcacgaacaa 3000
tgacgtgcaa gtaactgcca aacgtctaag tgcaggcaat gttgtttatg gcatcagcat 3060
gaatgacagt ggtagccgtg ttgcacaaaa cctagacaaa gtacttaatg atttagataa 3120
aaaacaagaa acacaagggt cactgaccag tgatgagaag caatttgcta accgtgtatt 3180
cactggtttt gaaaacatga attctgggtg agaattctaaa ctttctacag taagcaccaa 3240
ccgtgagcta tacaagcttg acccaacttt ctatgctgac agtgcattaa acgcagtaga 3300
agacagtgct aaccatgcaa ccgaatttgg taagcgtggt agcgcccaa gaggtgtttg 3360
gggtaatatc agtcaccatg attatgatgt agaactagag catgctacaa gtgcacgtaa 3420
aggcaacaac attagtgttg gtgcaagcac tcaaactgca gccgacatta gtgttggtgc 3480
acaacttgat gtaagtaaac ttgacttgga agaattctggt tatggtattg gcaacaaaac 3540
caaaactgac agcattggct tgactgttgg tgcttctaag aagttgggtg atgcctatct 3600
atcaggttgg gtaaaagggt ccaaagttga tacagaagcg aaccgtggtg aaaactctaa 3660
caaagttgag tacaatggta agctatatgg tgctggtatc caagcgggta caaacattga 3720
tactgcatcg ggcgtgagtg tacaacctta tgcttttgtt aaccatcagc agtacaaaaa 3780
cgatggtagc ttcaatgacg gtcttaacgt tggtagcgac atcgaagcaa aacaaactca 3840
ggtaggtgtg ggtgctgata tgggtgtcca agcaacacct gctctacagc ttactgggtg 3900
tgtgcaagtt gctcacgctg ttagccgtga caccaaccta gacactcgct atgttggtac 3960
agcgacagat gtacagtatg gcacttggga tactgacaaa accaaatggt cagccaaggt 4020
tggtgctaac tataatgtga caccaaacag ccaagtgggt ctaaaactaca gctacacagg 4080
tagtgcgcat tcagatgctt cccaagtggg tgtgagcttc accagcaagt tctaattcat 4140
taataaggca acaaaaaaca gcacaatttc ggttgtgctg ttttttgtga tgccgagcgt 4200
aaaattttcc caaaaaaagc gtgataatta ccacgctttt ttattgcata ttgcaaaaata 4260
gtattgcatt tatgggttgt taagcaaccc gtccaaatac cccctaaaca actccacccc 4320
aatcggtgct aacttgtttt gccacaggct cgtcaatgtg tcggcatcat caaccattac 4380
cgac

```

<210> 3

<211> 616

<212> PRT

<213> Moraxella bovis

<400> 3

```

Met Lys Lys Ser Ala Phe Ala Lys Tyr Ser Ala Leu Ala Leu Met Val
  1                      5                      10                      15

```

```

Gly Met Cys Leu His Thr Ala Tyr Ala Lys Glu Phe Ser Gln Val Ile
                20                      25                      30

```

```

Ile Phe Gly Asp Ser Leu Ser Asp Thr Gly Arg Leu Lys Asp Met Val
                35                      40                      45

```

```

Ala Arg Lys Asp Gly Thr Leu Gly Asn Thr Leu Gln Pro Ser Phe Thr
                50                      55                      60

```

```

Thr Asn Pro Asp Pro Val Trp Ser Ser Leu Phe Ala Gln Ser Tyr Gly
        65                      70                      75                      80

```

```

Lys Thr Ala Ser Ala Asn Thr Pro Tyr Asn Pro Thr Gly Thr Asn Tyr
                85                      90                      95

```

```

Ala Val Gly Gly Ala Arg Ser Gly Ser Glu Val Asn Trp Asn Gly Phe
                100                      105                      110

```

```

Val Asn Val Pro Ser Thr Lys Thr Gln Ile Thr Asp His Leu Thr Ala
                115                      120                      125

```

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Thr | Gly | Gly | Lys | Ala | Asp | Pro | Asn | Thr | Leu | Tyr | Ala | Ile | Trp | Ile | Gly |
| 130 | | | | | | 135 | | | | | 140 | | | | |
| Ser | Asn | Asp | Leu | Ile | Ser | Ala | Ser | Gln | Ala | Thr | Thr | Thr | Ala | Glu | Ala |
| 145 | | | | | 150 | | | | | 155 | | | | 160 | |
| Gln | Asn | Ala | Ile | Lys | Gly | Ala | Val | Thr | Arg | Thr | Val | Ile | Asp | Ile | Glu |
| | | | | 165 | | | | | 170 | | | | | 175 | |
| Thr | Leu | Asn | Glu | Ala | Gly | Ala | Thr | Thr | Ile | Leu | Val | Pro | Asn | Val | Pro |
| | | | 180 | | | | | 185 | | | | | 190 | | |
| Asp | Leu | Ser | Leu | Thr | Pro | Arg | Ala | Ile | Tyr | Gly | Glu | Ser | Leu | Met | Ala |
| | | 195 | | | | | 200 | | | | | 205 | | | |
| Gly | Val | Gln | Asp | Lys | Ala | Lys | Leu | Ala | Ser | Ser | Leu | Tyr | Asn | Ser | Gly |
| | 210 | | | | | 215 | | | | | 220 | | | | |
| Leu | Phe | Glu | Ala | Leu | Asn | Gln | Ser | Thr | Ala | Asn | Ile | Ile | Pro | Ala | Asn |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 |
| Thr | Phe | Ala | Leu | Leu | Gln | Glu | Ala | Thr | Thr | Asn | Lys | Glu | Ala | Phe | Gly |
| | | | | 245 | | | | | 250 | | | | | 255 | |
| Phe | Lys | Asn | Thr | Gln | Gly | Val | Ala | Cys | Gln | Met | Pro | Ala | Arg | Thr | Thr |
| | | | 260 | | | | | 265 | | | | | 270 | | |
| Gly | Ala | Asp | Asp | Val | Ala | Ser | Thr | Ser | Leu | Ala | Cys | Thr | Lys | Ala | Asn |
| | | 275 | | | | | 280 | | | | | 285 | | | |
| Leu | Ile | Glu | Asn | Gly | Ala | Asn | Asp | Thr | Tyr | Ala | Phe | Ala | Asp | Asp | Ile |
| | 290 | | | | | 295 | | | | | 300 | | | | |
| His | Pro | Ser | Gly | Arg | Thr | His | Arg | Ile | Leu | Ala | Gln | Tyr | Tyr | Arg | Ser |
| 305 | | | | | 310 | | | | | 315 | | | | | 320 |
| Ile | Met | Asp | Ala | Pro | Thr | His | Met | Gly | Lys | Leu | Ser | Gly | Glu | Leu | Val |
| | | | | 325 | | | | | 330 | | | | | 335 | |
| Lys | Thr | Gly | Ser | Ala | His | Asp | Arg | His | Val | Tyr | Arg | Gln | Leu | Asp | Arg |
| | | | 340 | | | | | 345 | | | | | 350 | | |
| Leu | Ser | Gly | Ser | Gln | His | Ser | Ile | Trp | Ala | Asn | Val | His | Ala | Ser | Asp |
| | | 355 | | | | | 360 | | | | | 365 | | | |
| Arg | Thr | Asp | Pro | Thr | Thr | Gln | Ile | Gly | Leu | Asp | Val | Ala | Gly | Ser | Ser |
| | | | | | | 375 | | | | | 380 | | | | |
| Ser | His | Thr | Gly | Ala | Tyr | Leu | Ser | His | Gln | Asn | Gln | Asp | Tyr | Val | Leu |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 |
| Asp | Asp | Thr | Leu | Ser | Ser | Asp | Val | Lys | Thr | Ile | Gly | Met | Gly | Leu | Tyr |
| | | | | 405 | | | | | 410 | | | | | 415 | |
| His | Arg | His | Asp | Ile | Gly | Asn | Val | Arg | Leu | Lys | Gly | Val | Ala | Gly | Ile |
| | | | | 420 | | | | 425 | | | | | 430 | | |

| | | | | | | |
|------------|-------------|------------|-------------|-------------|-------------|-----|
| <400> 4 | | | | | | |
| tgacaaataa | ttgggcattg | ggcagataac | ccatcaaaga | cccaaagcaa | cccataaatc | 60 |
| aaaaaaacac | ttgtaatttt | tgtaatatct | tgttacactt | tacaagtgtt | tttactttga | 120 |
| aagcaactca | gagagtaata | atgaaaaaat | cgcgctttgc | caaatactca | gcacttgccc | 180 |
| taatggttg | ggtgtgcctg | cacaccgctt | acgccaaagga | gtttagccaa | gtcatcattt | 240 |
| ttggggacag | cttgtccgat | acaggtcgcc | taaaagatat | ggtcgcccga | aaagatggca | 300 |
| cccttggcaa | caccttacag | ccatctttta | ccaccaaccc | cgaccttgta | tggtcaggct | 360 |
| tatttgccca | aagttatggc | aaaaccgcc | gtgccaacac | gccctacaat | ccactggcca | 420 |
| ctaactatgc | cgtgggcgga | gctcgctctg | ctcgcgaggt | caattggaat | ggtttttgtga | 480 |
| atgtaccctc | cacccaaaacg | caaatcaccg | accattttgac | cgccacaggt | ggcaaagccg | 540 |
| accctaatac | cctgtatgcc | atttggattg | gctctaatga | cttaatttca | gcttctcaag | 600 |
| ccaccacaac | agccgaagcc | caaaacgcc | ttaaagggtgc | ggtaactcgc | accgtgatag | 660 |
| acatcgaaac | actcaatcaa | gcagggcgga | caaccatttt | ggtgccaaat | gtgcctgatt | 720 |
| tgaqcttcac | gccccgagcc | atctatggcg | aaagcctcat | ggcaggcggtg | caagacaaag | 780 |

```

<400> 5
Met Ser Asn Ile Asn Val Ile Lys Ser Asn Ile Gln Ala Gly Leu Asn
  1          5          10          15
Ser Thr Lys Ser Gly Leu Lys Asn Leu Tyr Leu Ala Ile Pro Lys Asp
          20          25          30
Tyr Asp Pro Gln Lys Gly Gly Thr Leu Asn Asp Phe Ile Lys Ala Ala
          35          40          45
Asp Glu Leu Gly Ile Ala Arg Leu Ala Glu Glu Pro Asn His Thr Glu
  50          55          60
Thr Ala Lys Lys Ser Val Asp Thr Val Asn Gln Phe Leu Ser Leu Thr
  65          70          75          80
Gln Thr Gly Ile Ala Ile Ser Ala Thr Lys Leu Glu Lys Phe Leu Gln
          85          90          95
Lys His Ser Thr Asn Lys Leu Ala Lys Gly Leu Asp Ser Val Glu Asn
          100          105          110
Ile Asp Arg Lys Leu Gly Lys Ala Ser Asn Val Leu Ser Thr Leu Ser
          115          120          125
Ser Phe Leu Gly Thr Ala Leu Ala Gly Ile Glu Leu Asp Ser Leu Ile
  130          135          140

```

| | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|
| Lys | Lys | Gly | Asp | Ala | Pro | Asp | Ala | Leu | Ala | Lys | Ala | Ser | Ile | Asp | | | |
| 145 | | | | | 150 | | | | | 155 | | | | 160 | | | |
| Leu | Ile | Asn | Glu | Ile | Ile | Gly | Asn | Leu | Ser | Gln | Ser | Thr | Gln | Thr | Ile | | |
| | | | | 165 | | | | | 170 | | | | | 175 | | | |
| Glu | Ala | Phe | Ser | Ser | Gln | Leu | Ala | Lys | Leu | Gly | Ser | Thr | Ile | Ser | Gln | | |
| | | | 180 | | | | | 185 | | | | | 190 | | | | |
| Ala | Lys | Gly | Phe | Ser | Asn | Ile | Gly | Asn | Lys | Leu | Gln | Asn | Leu | Asn | Phe | | |
| | | 195 | | | | | 200 | | | | | 205 | | | | | |
| Ser | Lys | Thr | Asn | Leu | Gly | Leu | Glu | Ile | Ile | Thr | Gly | Leu | Leu | Ser | Gly | | |
| | 210 | | | | | 215 | | | | | 220 | | | | | | |
| Ile | Ser | Ala | Gly | Phe | Ala | Leu | Ala | Asp | Lys | Asn | Ala | Ser | Thr | Gly | Lys | | |
| 225 | | | | | 230 | | | | | 235 | | | | | 240 | | |
| Lys | Val | Ala | Ala | Gly | Phe | Glu | Leu | Ser | Asn | Gln | Val | Ile | Gly | Asn | Val | | |
| | | | | 245 | | | | | 250 | | | | | 255 | | | |
| Thr | Lys | Ala | Ile | Ser | Ser | Tyr | Val | Leu | Ala | Gln | Arg | Val | Ala | Ala | Gly | | |
| | | | 260 | | | | | 265 | | | | | 270 | | | | |
| Leu | Ser | Thr | Thr | Gly | Ala | Val | Ala | Ala | Leu | Ile | Thr | Ser | Ser | Ile | Met | | |
| | | 275 | | | | | 280 | | | | | 285 | | | | | |
| Leu | Ala | Ile | Ser | Pro | Leu | Ala | Phe | Met | Asn | Ala | Ala | Asp | Lys | Phe | Asn | | |
| | 290 | | | | | 295 | | | | | 300 | | | | | | |
| His | Ala | Asn | Ala | Leu | Asp | Glu | Phe | Ala | Lys | Gln | Phe | Arg | Lys | Phe | Gly | | |
| 305 | | | | | 310 | | | | | 315 | | | | | 320 | | |
| Tyr | Asp | Gly | Asp | His | Leu | Leu | Ala | Glu | Tyr | Gln | Arg | Gly | Val | Gly | Thr | | |
| | | | | 325 | | | | | 330 | | | | | 335 | | | |
| Ile | Glu | Ala | Ser | Leu | Thr | Thr | Ile | Ser | Thr | Ala | Leu | Gly | Ala | Val | Ser | | |
| | | | 340 | | | | | 345 | | | | | 350 | | | | |
| Ala | Gly | Val | Ser | Ala | Ala | Ala | Val | Gly | Ser | Ala | Val | Gly | Thr | Pro | Ile | | |
| | | 355 | | | | | 360 | | | | | 365 | | | | | |
| Ala | Leu | Leu | Val | Ala | Gly | Val | Thr | Gly | Leu | Ile | Ser | Gly | Ile | Leu | Glu | | |
| | 370 | | | | | 375 | | | | | 380 | | | | | | |
| Ala | Ser | Lys | Gln | Ala | Met | Phe | Glu | Ser | Val | Ala | Asn | Arg | Leu | Gln | Gly | | |
| 385 | | | | | 390 | | | | | 395 | | | | | 400 | | |
| Lys | Ile | Leu | Glu | Trp | Glu | Lys | Gln | Asn | Gly | Gly | Gln | Asn | Tyr | Phe | Asp | | |
| | | | | 405 | | | | | 410 | | | | | 415 | | | |
| Lys | Gly | Tyr | Asp | Ser | Arg | Tyr | Ala | Ala | Tyr | Leu | Ala | Asn | Asn | Leu | Lys | | |
| | | | 420 | | | | | 425 | | | | | 430 | | | | |
| Phe | Leu | Ser | Glu | Leu | Asn | Lys | Glu | Leu | Glu | Ala | Glu | Arg | Val | Ile | Ala | | |
| | | | 435 | | | | 440 | | | | | 445 | | | | | |

WO 01/16172

PCT/AU00/01048

11/14

Ile Thr Gln Gln Arg Trp Asp Asn Asn Ile Gly Glu Leu Ala Gly Ile
450 455 460

Thr Lys Leu Gly Glu Arg Ile Lys Ser Gly Lys Ala Tyr Ala Asp Ala
465 470 475 480

Phe Glu Asp Gly Lys Lys Val Glu Ala Gly Ser Asn Ile Thr Leu Asp
485 490 495

Ala Lys Thr Gly Ile Ile Asp Ile Ser Asn Ser Asn Gly Lys Lys Thr
500 505 510

Gln Ala Leu His Phe Thr Ser Pro Leu Leu Thr Ala Gly Thr Glu Ser
515 520 525

Arg Glu Arg Leu Thr Asn Gly Lys Tyr Ser Tyr Ile Asn Lys Leu Lys
530 535 540

Phe Gly Arg Val Lys Asn Trp Gln Val Thr Asp Gly Glu Ala Ser Ser
545 550 555 560

Lys Leu Asp Phe Ser Lys Val Ile Gln Arg Val Ala Glu Thr Glu Gly
565 570 575

Thr Asp Glu Ile Gly Leu Ile Val Asn Ala Lys Ala Gly Asn Asp Asp
580 585 590

Ile Phe Val Gly Gln Gly Lys Met Asn Ile Asp Gly Gly Asp Gly His
595 600 605

Asp Arg Val Phe Tyr Ser Lys Asp Gly Gly Phe Gly Asn Ile Thr Val
610 615 620

Asp Gly Thr Ser Ala Thr Glu Ala Gly Ser Tyr Thr Val Asn Arg Lys
625 630 635 640

Val Ala Arg Gly Asp Ile Tyr His Glu Val Val Lys Arg Gln Glu Thr
645 650 655

Lys Val Gly Lys Arg Thr Glu Thr Ile Gln Tyr Arg Asp Tyr Glu Leu
660 665 670

Arg Lys Val Gly Tyr Gly Tyr Gln Ser Thr Asp Asn Leu Lys Ser Val
675 680 685

Glu Glu Val Ile Gly Ser Gln Phe Asn Asp Val Phe Lys Gly Ser Lys
690 695 700

Phe Asn Asp Ile Phe His Ser Gly Glu Gly Asp Asp Leu Leu Asp Gly
705 710 715 720

Gly Ala Gly Asp Asp Arg Leu Phe Gly Gly Lys Gly Asn Asp Arg Leu
725 730 735

Ser Gly Asp Glu Gly Asp Asp Leu Leu Asp Gly Gly Ser Gly Asp Asp
740 745 750

| <400> 6 | | | | | | |
|-------------|-------------|------------|------------|-------------|-------------|-----|
| atgagaacgt | tatttttcaga | tgaattgttt | agagcgattc | gtgtagatgg | aaatttcatcg | 60 |
| catggttaaga | tatctgaatt | ttagtgaaag | tctgttgatt | caaaattagc | ctcaagaata | 120 |
| tttgcacaaat | atcacgaaga | tttgacgagc | aaattgtcaa | ctcagaataa | ttttattata | 180 |
| tctaaagata | attaatacaa | ccttttctaa | caacaacgag | agagacatat | tatgtccaat | 240 |
| ataaatgtaa | ttaaattctaa | tattcaagca | ggcttgaatt | caacaaagtc | tggattaaaa | 300 |
| aatctttact | tggctattcc | caaagattat | gatccgcaaa | aagggtggag | tttaaatgat | 360 |
| tttattaaag | ctgctgatgt | attaggattt | gctcgtttag | cagaaggagcc | taatcacact | 420 |
| gaaacagcaa | aaaaactctg | tgacacagta | aatcagtttc | tctctctcac | acaaactcgt | 480 |
| attgctatttt | ctgcaacaaa | attagaaaag | ttcttacaaa | aacattctac | caataagtta | 540 |
| gccaaagggg | tagacagtgt | agaaaatatt | gacgtaaat | taggtaaagc | aagtaatgta | 600 |
| ttatcaacat | taagctcttt | tttgggcact | gcattagcgg | gtatagaact | tgattcttta | 660 |
| atcaaaaaag | gtgatgctgc | acctgatgct | ttggctaaag | ctagtattga | cttgattaat | 720 |
| gagataattg | gtaattctatc | tcagagtact | caaacgattt | aagcattttc | ttcacagtta | 780 |
| gcaaaagttg | gttctactat | atcgcaggct | aaaggcttct | ctaatatagg | aaacaagttg | 840 |
| caaaacttaa | atttttctaa | aacaaatcct | ggtttggaag | taattactgg | tttgctatca | 900 |

13/14

```

ggcattttctg caggcttttgc ttttagcggat aaaaatgcat cgactggcaa aaaagttgct 960
gcaggtttttg aattaagcaa tcaagttatt ggtaatgtaa caaaagcaat ttcttcatat 1020
gttttagcac aacgtgttgc tgctgggtcta tcaactactg gtgctgttgc tgctttaatt 1080
acttcacgca ttatgttggc aattagtcct ttggcattta tgaatgcagc agataaaattc 1140
aatcatgcta atgctcttga tgagtttgca aaacaattcc gaaaatttgg ctatgatggg 1200
gatcatttat tggctgaata tcagcgtggg gtgggtacta ttgaagcttc attactaca 1260
attagtagcg cattaggtgc agtttctgct ggtgtttccg ctgctgctgt aggatctgct 1320
gttggtacac cgattgcaat attagttgca ggtgttacag gattgatctc tggaatttta 1380
gaagcgtcta aacaggcaat gtttgaaagt gttgctaacc gtttacaagg taaaatttta 1440
gagtgggaaa agcaaaatgg cggtcagaac tattttgata aaggctatga ttctcgttat 1500
gctgcttatt tagctaataa cttaaaattt ttgtctgagc taaataaaga gttggaagct 1560
gaacgtgtta ttgcaatcac ccaacaacgt tgggataata atattgggtga gttagcagg 1620
attaccaaatt tgggtgaacg cattaagagc ggaaaagctt atgcagatgc ttttgaagat 1680
ggcaagaaaag ttgaagctgg ttccaatatt actttggatg ctaaaactgg tatcatagac 1740
attagtaatt caaatgggaa aaaaacgcaa gcgttgcat tcaactcgcc tttgttaaca 1800
gcaggaactg aatcacgtga acgtttaact acatgtaaat actcttatat taataagtta 1860
aaattcggac gtgtaaaaaa ctggcaagtt acagatggag aggctagttc taaattagat 1920
ttctctaagg ttattcagcg tgtagccgag acagaaggca cagacgagat tgggtctaata 1980
gtaaatgcaa aagctggcaa tgacgatatc tttgttggtc aaggtaaaat gaattattgat 2040
ggtggagatg gacacgatcg tgtcttctat agtaaagacg gaggatttgg taattattact 2100
gtagatggta cgagtgaac agaagcaggc agttatacag ttaatcgtaa ggttgctcga 2160
ggtgatattc accatgaagt tgtgaagcgt caagaaacca aggtgggtaa acgtactgaa 2220
actatccagt atcgtgatta tgaattaaga aaagttgggt atggttatca gtctaccgat 2280
aatttgaaat cagtagaaga agtaattggg tctcaattta atgatgtatt caaaggttct 2340
aaattcaacg acatattcca tagtggtgaa ggtgatgatt tactcgatgg tgggtgctgg 2400
gacgaccgct tgtttggtgg taaaggcaac gatcgacttt ctggagatga aggcgatgat 2460
ttactcgatg gcggttctgg tgatgatgta ttaaatgggt gtgctggtaa tgatgtctat 2520
atctttcggg aaggtgatgg taatgatact ttgtacgatg gcacgggcaa tgataaatta 2580
gcatttgcag atgcaaatat atctgatatt atgattgaac gtaccaaaga gggattata 2640
gttaaacgaa atgatcattc aggtagtatt aacataccaa gatggtacat aacatcaaat 2700
ttacaaaatt atcaaagtaa taaaacagat cataaaattg agcaactaat tggtaaagat 2760
ggtagttata tcaattccga tcaaattgat aaaattttgc aagataagaa agatgggtaca 2820
gtaattacat ctcaagaatt gaaaaagctt gctgatgaga ataagagcca aaaattatct 2880
gcttcggaca ttgcaagtag cttaaaataag ctagttgggt caatggcact atttgggtaca 2940
gcaaatagtg tgagttctaa cgccttacag ccaattacac aaccaactca aggaattttg 3000
gctccaagtg tttagtgatt taattttact gacaatatca ccacccatat cattgggtat 3060
agattatgaa actagtgata tgggtgggtga tacttcttta attagactta atttacaaac 3120
ccttaatatg aatttagtta tgatagatta tgctcaacaa cctgctctat ctgctctggt 3180
tatecttgcc aaatactatg gtatttctgc aagtccagca gacattatgc a 3231

```

<210> 7
 <211> 17
 <212> PRT
 <213> Moraxella bovis

<220>
 <221> misc_feature
 <222> (14) (16)
 <223> Xaa = unknown

<400> 7
 Lys Glu Phe Ser Gln Val Ile Ile Phe Gly Asp Ser Leu Xaa Asp Xaa
 1 5 10 15

```

<400> 9
Met Gly Gly Asp Thr Ser Leu Ile Arg Leu Asn Leu Gln Thr Leu Asn
  1                               10                               15

Ser Asn Leu Val Met Ile Asp Tyr Ala Gln Gln Pro Ala Leu Ser Ala
      20                               25                               30

Leu Val Ile Leu Ala Lys Tyr Tyr Gly Ile Ser Ala Ser Pro Ala Asp
      35                               40                               45

Ile Met His Arg Leu Ala Lys Lys Leu
      50                               55

```